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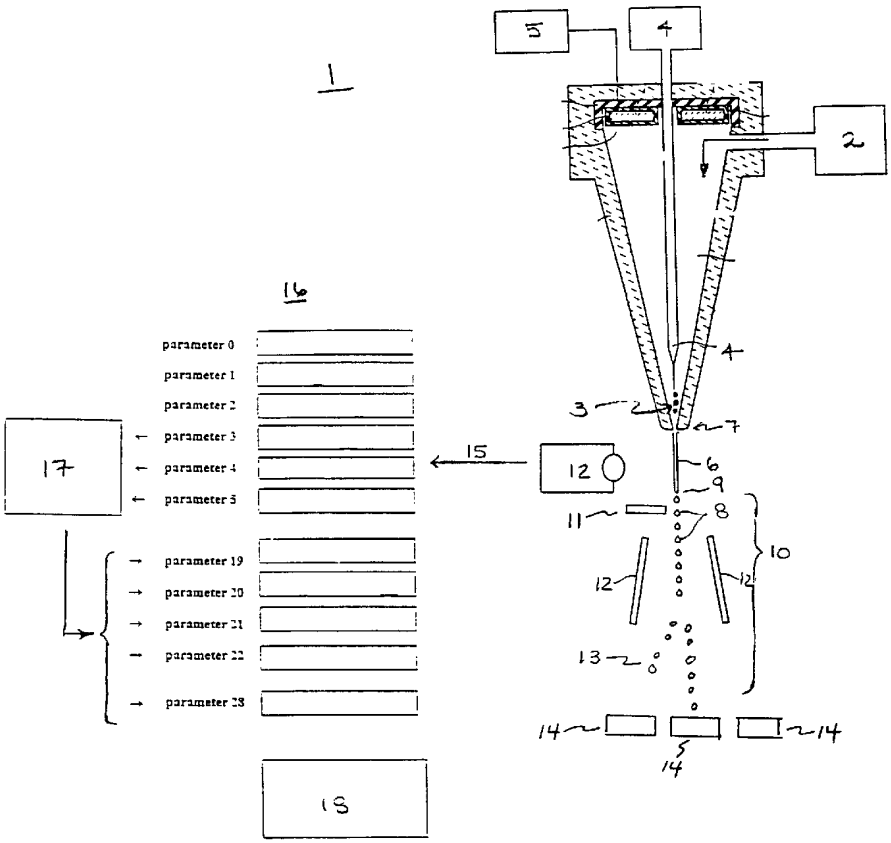

(57) Abstract: A flow cytometry apparatus (1) and methods to process information incident to particles or cells entrained in a sheath fluid stream allowing assessment, differentiation, assignment, and separation of such particles or cells even at high rates of speed. A first signal processor (18) individually or in combination with at least one additional signal processor (17) for applying compensation transformation on data from a signal (15). Compensation transformation can involve complex operations on data from at least one signal (15) to compensate for one or numerous operating parameters. Compensated parameters can be returned to the first signal processor (18) for providing information upon which to define and differential particles (3) from on another.

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TRANSIENTLY DYNAMIC FLOW CYTOMETER ANALYSIS SYSTEM

I. TECHNICAL FIELD

Specifically, flow cytometry apparatus and methods to process information incident to particles or cells entrained in a sheath fluid stream allowing assessment, differentiation, assignment, and separation of such particles or cells even at high rates of speed.

II. BACKGROUND

Flow cytometry is a field which has existed for many years. Basically, flow cytometer systems act to position small amounts of a substance within a sheath fluid. Through hydrodynamic focusing and laminar flow, the substance is split into individual particles, cells, or the like. In many applications, sheath fluid together with its entrained substance exits a nozzle in a jet and free falls or is channeled in an optically transparent pathway for analysis. The sheath fluid may form droplets encapsulating individual particles which are separated and collected based upon assignment of differentiated particle characteristics.

This type of analysis requires uniform conditions within the jet, very precise timing, and consistent comparative parameters incident to the entrained substances to separate such substances accurately. In addition, there is a coincident commercial and public sector demand for higher speed flow cytometry, the need to differentiate substances based on more complex and multiple parameter analysis, and for higher purity separation(s). Unfortunately, variation in equipment operation, sheath fluid stream dynamics, or observed particle characteristics still exists and are exacerbated by increasing the speed at which entrained substances are carried in the jet. As such, there is a need to compensate for such variations to provide for accurate analysis and separation of the substances entrained in the sheath fluid stream.

An overview of some attempts to understand and react to fluid stream and droplet dynamics can be seen in United States Patents Nos. 4317520, 4318480, 4318481, 4318482, 4318483, and 4325483, each hereby incorporated by reference herein. As these explain, traditionally the approach has been to assess the signals and act directly upon such information. Some of the practical problems which have also been recognized is the fact that only a limited amount of space and time exists within which to conduct sensing and analysis.

As Japanese Patent 2024535 also recognizes with respect to the sensing system alone, it may be desirable to have an optical system which is as small as possible.

As can be understood, a substantial problem can be that the data generated from an occurrence must be sensed and reacted upon in an extremely short period of time. Given the speed of microprocessors and the like, this might, at first glance, appear to be readily achievable. The challenge for this unique flow cytometry situation is that original or raw signal data can be sub-optimal and even unusable. As such, if it is to be used, it must be further processed in order to accomplish further analysis or decision making. This processing can be complex and can require more processing speed and power than is available not just with typical commercial systems, but even with today's highest-speed computer systems. Further, as the desire for higher processing frequencies is pursued, problems can be compounded. An example of the extremes to which speed has been taken is shown in United States Patent No. 4361400, hereby incorporated by reference herein, where droplet formation frequencies in the range of 300 to 800 kilohertz had been achieved. Most practical droplet flow cytometers operate in the range of 10 to 50 kHz. Although speed of analysis problems have been known for years, prior to the present invention it has apparently been an accepted attitude that digital analysis in the flow cytometry context could not be achieved. This invention proves this expectation to be untrue. As a result of the present invention, droplet formation speeds in the 50-100-200 or higher kHz ranges are now possible with adequate data compensation and the like.

At any of these speeds, however, there appears to has been an expectation that analog analysis was the only practical way to achieve analysis of and to compensate for fluid dynamics, particle characteristics, equipment variance, and the like. To some degree, these expectations have been so prevalent that quality control, good manufacturing practices, regulatory approval, and other concerns have been set aside, diminished, or even compromised. The previously existing technology governing the practices of those in this field.

Another significant problem associated with conventional analysis and compensation of variables in flow cytometry can be the preservation of original signal data from an

occurrence incident to the fluid stream prior to subsequent processing steps. It may not have been possible to preserve or store original signal data until now due to the short amount of time in which to analyze or compensate the original signal. As such, all or part of the original or raw signal data may have been sacrificed to increase the efficiency of analysis or provide feed back compensation events. The practice of discarding original raw data may prevent re-analysis of the data to improve quality control, to establish good manufacturing practices, and attain procedural thresholds for certain regulatory or statutory requirements.

Yet another problem with conventional analysis may be the inability to process high speed serial occurrences, to compensate multiple parameters, to perform complex operations, to provide transformation compensation of original data, or to apply compensated parameters. Conventional analysis can be limited by the amount of information that can be processed and returned in between serial events which can occur at a rates of at least 10,000 per second.

A first aspect of this inability can be associated with the nature of conventional signal processors used with flow cytometry. Conventional flow cytometer signal processors, often because they are analog, are not capable of dealing with large amounts of signal information, cannot perform operations on low quality signal information, cannot practically accomplish complex transformation operations (such as those which use algebraic expressions or structure), or they perform only reflexive feed back operations rather than serial or multi-variant analysis followed by subsequent parameter compensation.

A second aspect of this inability can be associated with the infrastructure of conventional data handling. In part, conventional infrastructure may not deal with how the streams of information are allocated, aligned, and coordinated. Conventional processing of flow cytometer information from occurrences incident to the fluid stream are traditionally handled as isolated feedback loops. As such, it can become increasingly difficult to synchronize various aspects of flow cytometer operation as the number of feed back loops increases. Moreover, these feed back loops may be completely uncoupled. For example, stream parameters, such as droplet break off location, may be completely uncoupled from the differential analysis of and separation of particles within the fluid stream being compensated.

A third aspect of this inability may be lack of symmetry reduction in the application of transformed data. Again, analog analysis can prevent or minimize symmetry reduction in the complex analysis of serial occurrences or parallel multivariant analysis. The lack of symmetry reduction or the inability to apply symmetry reduction to analysis terms may increase execution time.

As mentioned above, there has been a long felt but unsatisfied need for apparatus and methods which permit complex signal transformation, and use of compensated parameters resulting from complex signal transformation, real time analysis using compensated paramenters, or storage of original signal data generated incident to the fluid stream, instrument variance, or environmental variance. The present invention addresses each of the above-mentioned problems with a practical solution. To some extent, it is apparent that solutions have not been achieved because those skilled in the art seem to have taken a direction which was away from the technical direction pursued in the present invention. This may have been the result of the fact that those skilled in the art did not truly appreciate the nature of the problem or it may have been the result of the fact that those skilled in the art were misled by some of the presumptions and assumptions with respect to the type of systems which could be considered. The present invention uses digital signal processing (DSP) technology to structure information from occurrences incident to flow cytometer operation, and to perform complex transformation, compensation, or analysis operations to achieve this long sought goal.

III. DISCLOSURE OF THE INVENTION

The present invention discloses a flow cytometer having DSP technology to solve problems associated with high speed serial occurrences, or multiple parameter analysis of occurrences, or both individually or in combination. While specific examples are provided in the context of flow cytometry applications to illustrate the invention, this is not meant to limit the scope of the invention to that field or to applications within flow cytometry. As such, the invention may also have numerous applications in various fields, for example, detection of defects in products as disclosed by United States Patent Nos. 4,074,809 and 4,501,366; field flow fractionation, liquid chromatography, or electrophoresis as disclosed by United States Patent No. 5,503,994; computer tomography, gamma cameras, or time of flight instruments

as disclosed by United States Patent No. 5,880,457, each of the above-mentioned patents are hereby incorporated by reference herein. It should be understood that the basic concepts of the invention may be applied not only to the area of flow cytometry but may apply to each of the above mentioned fields, or to other fields where the detection and analysis of small differences in parameters, such as photo-generated signal between serial occurrences having high incident light flux, or serial occurrences generating data concerning multiple parameters, or occurrences that generate a high number of signals in a short period of time, may be necessary or desired. Moreover, it should be understood that the invention can be divided into a number of embodiments which may be combined in various permutations and combinations. Naturally, as a result of these several different and potentially independent aspects of the invention, the objects of the invention are quite varied.

One broad object of an embodiment of the invention can be to convert original signals incident to the environment, the instrument, or a fluid stream, including but not limited to analog signals, to digital signals. One aspect of this object of the invention can be to harmonize a plurality of different types of signals into a fresh digitized data stream for processing. Another aspect of this object of the invention be to convert otherwise low quality or unusable signal data into usable quality signal data. In this regard, the original signal could be associated with a characteristic or multiple characteristics of single particle, such as a cell, within a fluid stream. Alternately, the original signal could be associated with a characteristic or multiple characteristics of a series of particles within a fluid stream. As such, numerous signals may be generated from the sensing of simultaneous occurrences (parallel occurrences) or the sensing of discrete occurrences over time (serial occurrences) that represent one, two, or any number of additional parameters. The rate of occurrences sensed may vary between about 10,000 occurrences per second to about 800,000 occurrences per second or more. The occurrences may be, as examples, the change in fluid dynamics at the jet or nozzle, the variation of in performance of the equipment itself (such as the change in the baseline electronic signal from a photomultiplier tube), or the variation in performance of equipment due to the change in external conditions such as temperature or pressure. As to each, the occurrence, even when occurring at a high rate, or occurring for a limited duration, or occurring in a sub-optimal manner may be sensed, converted to an original signal, and digitized.

Another broad object of an embodiment of the invention can be to perform compensation transformation on the original signal to provide compensated parameters. One aspect of this object can be to apply compensation transformation to processed data from a first signal incident to a first occurrence and to then apply compensation transformation to processed data from at least one additional signal incident to one or more occurrences to compensate a parameter(s) shared by the first occurrence and by at least one additional occurrence. A second aspect of this object can be compensation of parameter(s) that share characteristic(s) so that "cross talk" can be eliminated or minimized. Elimination or minimization of crosstalk provides an increased ability to differentiate a first occurrence from a second or more occurrence(s). Differentiated occurrences may then be assigned to a class, separated, and collected.

Another object of an embodiment of the invention is to provide hardware or software infrastructure to allocate, align, or coordinate data generated from the above-mentioned original signals. One aspect of this object can be to provide multiple signal processors that can operate in parallel to increase the capacity to process signal data. The instant invention can utilize at least two but could utilize many parallel signal processors. The parallel signal processors could be stand aside hardware, or hardware that can coupled together via ether-net or Internet connections. A second aspect of this object of the invention can be to allocate different functions to the various parallel signal processors so as to optimize processing speed. A third aspect of this object of the invention can be to use linear assemblers and register usage to enhance parallel operation of and to coordinate the specialized functions performed by at least two signal processors. A fourth aspect of this object can be to provide software which optimizes the use of parallel processing of digital code. A fifth aspect of this object of the invention can be to apply symmetry reduction to serial transformation operations to reduce processing execution time.

Another object of an embodiment of the invention can be to perform complex operations on the above-mentioned original signals. Complex operations can be operations that were not possible or were not practical prior to the invention due to the speed at which the operations have to be performed in serial or in parallel, the number of parameters involved, the utilization of algebraic expressions or structure, the use of complex numbers to define

variables, or the like. Each of these aspects can be complex individually or complex in combination.

Another object of an embodiment of the invention can be to save the original signal in a memory element or memory storage element. One aspect of this object can be to save the original signal without altering the original quality or quantity of the original signal. This may be necessary or desirable for quality control concerns or to meet regulatory or statutory requirements. Another aspect of this object can be to duplicate the original signal for analysis during flow cytometer operation or to duplicate the signal for future re-analysis.

Another object of an embodiment of the invention can be to provide software to implement the various applications on DSP technology. A first aspect of this object can be to provide exemplary compensation transformation operations. This may include compensation transformation for two way compensation, three way compensation, and so on for higher order compensation sets. A second aspect of this object can be to provide exemplary compensation matrices and their various properties. A third aspect of this object can be to provide exemplary symmetry reduction in various aspects of the software notation. A fourth aspect can be to provide an exemplary program for the subtraction of pairs or groups of fluorescent signals in order to orthogonalize the color sensitivity of each signal.

Yet another object of an embodiment of the invention can be to provide analog to digital converter compensation of amplified photomultiplier tube (PMT) outputs. Since emission spectra of fluorescent antibody labels is broadband, they can overlap the passbands of up to eight photomultiplier filters. Therefore, a digitized PMT output from even one antibody label can contain the effects of as many as eight antibody labels. See Shapiro, "Practical Flow Cytometry", pp. 17-19, 163-166 (19), hereby incorporated by reference herein. This feature allows color sensitivity to be orthogonalized for each signal, and specifically allows for the application in the context of the MoFlo® flow cytometer.

Yet another object of an embodiment of the invention can be to provide the ability to latch numerous parameters either simultaneously or interchangeably, and to specifically latch any of the maximum of sixty-four MoFlo® flow cytometers parameters as inputs.

Another object of an embodiment of the invention can be to provide cross beam time alignment in order to perform enhanced compensation between a pair of parameters. One aspect of this object can be to reduce the apparent inter-beam transition time to not more than 1 part in 3000 or to a compensated beam to beam "time jitter" of not more than one nanosecond which appears to be beyond the practical capability of analog circuit design.

Another object of an embodiment of the invention can be to provide digital error compensation. Digital substraction is attractive because it avoids the problems of signal alignment, however, major digitalization errors can occur. For example, when bright signals are compensated over a large dynamic range digitized errors, which can be visually discernable as a picket-fence coarseness of the compensated population, can occur. Digital error compensation can minimize these errors and hence improve the quality of the digital information.

Another object of an embodiment of the invention can be to provide log amplifier idealization. Typically log amplifiers vary from ideal logarithmic behavior throughout their entire range. For example, some log amplifiers have a 0.4 db variance. That is, for any given input, the ratio of the output signal from a practical log amplifier over the value expected of a perfect logarithmic function is expressed in db as:

$$\text{Error} = 0.4 \text{ db} = 20\log10(\text{Vout}/\text{Videal})$$

Log amplifier idealization can provide values which more closely approximate the ideal amplifier.

Another object of an embodiment of the invention can be to provide off-loaded binning. The characteristics of , for example, populations of particles can be stored in the memory of a an additional signal processor using binning transformations. The statistical characterization of these populations, such as mean, standard deviation, skewness and separation can be sent to a separate processor, thus off-loading this task and hence increasing the performance of the first processor and the separate processor.

Naturally, further independent objects of the invention are disclosed throughout other areas of the specification.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic cross sectional view of a flow cytometer embodiment of the invention showing the various features combined.

Figure 2 shows a hardware schematic of an embodiment of the invention.

V. MODE(S) FOR CARRYING OUT THE INVENTION

Specifically, an enhanced flow cytometer utilizing DSP technology and methods to process raw or original signal information incident to various parameters during operation, including, but not limited to, environmental parameters, instrument parameters, or parameters incident to the particles or cells entrained in a sheath fluid stream allowing for complex assessment, differentiation, assignment, and separation of such particles or cells, even when the flow cytometer is operated at high speed. Generally, a data acquisition, data transformation, parameter compensation, and compensated parameter utilization system for the differentiation, assignment, and separation of multiple parallel or serial events that can be useful in numerous fields and applications.

In discussing these aspects of the invention some references may be made to MoFlo® (a trademark of Cytomation, Inc.) flow cytometer systems and Summit® (also a trademark of Cytomation, Inc.) capabilities for such systems. Each of these systems represent state-of-the-art flow cytometry capabilities which are not only the fastest practical flow cytometer systems, but they also are well known to those of ordinary skill in the art.

Referring now to Figure 1, a preferred embodiment of the invention can be seen in detail. A flow cytometer (1) having a fluid stream source (2) can establish a fluid stream into which particles (3) can be suspended. The source of particles (4) can insert the particles from time to time such that at least one particle becomes suspended in and is hydrodynamically focused in the stream. An oscillator (5) responsive to the fluid stream perturbs the fluid stream. A jet or fluid stream (6) comprised of the fluid stream (2) and the particles (3) can

then be established below the tip of the nozzle (7) of the flow cytometer. The stream can be established in a steady state condition such that droplets (8) that encapsulate a single particle form and break away from the contiguous part of the stream. When the stream is established in this steady state fashion, a stable droplet break-off point can be established. Below the droplet break-off point (9) a free fall zone (10) can exist. This free fall zone embodies the area where the droplets move once they break away from the contiguous part of the stream. A sensor (12), such as a laser and receiver in combination (or separately), can be used to monitor the stream for a particle. The sensor can sense an occurrence and generates a signal (15). For example, a coherent beam of light aimed at the fluid stream by the sensor (12) intercepts a particle (3) in the stream (6) and fluorescence or scattered light rays can then be emitted. The emitted fluorescence can be captured by the receiver, such as a photomultiplier tube, to generate the signal (15). Based upon analysis of the signal generated by the sensor from the flourescent occurrence, the particle(s) may be differentiated, and assigned to a class. A droplet charging location (11) can exist at a point along the free fall zone. Based upon the assignment of the particle, the droplet can be charged positively, negatively, or left uncharged.

As the charged droplets fall in the free fall zone, they can pass through an electrostatic field (12). If the droplets have been charged with a positive or negative charge, an electric field established between these electrostatic plates can deflect the charged droplets such that the trajectory of the deflected droplets (13) and the trajectory of the neutral droplets serves to separate one type of particle class from another. These separated particles can then be collected into a container(s) (14). Furthermore, alternative techniques such as utilizing different quantities of charge can be used to accomplish the assignment and separation of numerous classes of particles. The rate of separating the classes of particles or the sort rate can be at least 1000 per second.

The sensor (12) can be used to monitor or sense, and then assist in or generate a signal (15) incident to a variety of parameters (16) related to the operation of a flow cytometer or numerous other instruments (used individually or in combination). As described above, the raw or original signal(s) could be associated with a characteristic or multiple characteristics of a single particle (3), which could be a cell, entrained in the fluid stream (2). Alternately, the original signal could be associated with a characteristic or multiple characteristics of a

series of particles (3) or cells within the fluid stream (2). As further mentioned above, numerous signals may be generated from the sensing of simultaneous occurrences (parallel occurrences) or the sensing of discrete occurrences over time (serial occurrences) that represent one, two, or any number of additional parameters (at least 64 parameters in the MoFlo® flow cytometer). The rate of occurrences sensed may vary between few per second or could be between about 10,000 occurrences per second to about 800,000 occurrences per second, or even higher. The original signal may also represent, as examples, the change in fluid dynamics at the jet or nozzle, the variation in performance of the equipment itself (such as the change in the baseline electronic signal from a photomultiplier tube), or the variation in performance of equipment due to the change in external conditions such as temperature or pressure. Specifically, as shown in Figure 1 the parameters could be a variety of aspects incident to the fluorescent emission of fluorenylisothiocyanate (FITC) upon excitation and include pulse width, forward scatter, side scatter, raw FITC information, raw PE, raw PE-CYS, and so forth. Naturally, numerous other parameters could be also be monitored and these specific examples are meant to be used as a guide rather than an inclusive list.

The MoFlo® system, for example, monitors some conventional twelve bit parameters containing pulse width, analog to digital converter (ADC) channel outputs, timer outputs, Look Up Table (LUT) outputs, and the Classifier output. MoFlo® users can have need or desire to compute additional parameters which include compensating ADC outputs for the unwanted side effects of broadband fluorescence, computing ratios of ADC channel, and calculating whether ADC parameters fall inside, or outside 3D or higher dimensional regions, and the like. To expand the capability of instruments such as the MoFlo® system, other types of flow cytometer systems, or other types of instruments, the invention employs at least one additional signal processor (17) to apply compensation operations to the processed data from a first signal and to the processed data from a second or more signals. This may occur in parallel or simultaneous with the data processing of a first signal processor (18). The compensated output from the additional signal processor for at least one parameter shared by the signals (or the occurrences which generated the signals) allows enhanced differentiation between the first signal and the second signal based for the compensated parameter(s). The compensated data can then be combined into the data handling functions of the first signal processor, for example, and applied to classify and separate the occurrences.

Pass Through, Transformation and Return. Again referring to Figure 1, and as mentioned above, the data emerging from the flow cytometer may exploit at least one additional signal processor, that can for example, be a parallel digital signal processor (17) which may be used simultaneously with a first signal processor. The original raw data or a portion of the original raw data from each signal generated by the flow cytometer can be assembled as a table of 32 or more 16 bit data words. The first 16 data words could be the raw data outputs from an occurrence, such as fluorescent emissions from excited fluorochromes used as surface or internal markers. The first 16 data words may be passed through the additional signal processor and the transformed output may be then presented on the second (or more) 16 data words. The final compensated parameters are returned to the first signal processor, combined with the output of the first signal processor, and then presented or displayed. This is often referred to as a pass-through and return digital signal path.

Naturally, the numeric data formats for a particular application may have to be matched. For example the raw 12 bit MoFlo® data can be thought of as a unsigned fixed point integers, in the format 12.0, that is 12 integer bits to the left of the fixed point, and 0 fractional bits to the right of the fixed point. This yields a range of 0x000 (0) to 0xFFF (4095). The compensated parameter output from the second signal processor (17) may need to be in the same format. The internal data manipulations can be changed as required, to perform the required algorithms. Possible internal data formats that could be used are 2's complement, signed integer, signed or unsigned fractional fixed point numbers, or floating point decimal, as examples. Various CPLD/FPGA or digital signal processing Von Neuman or Harvard program, data, and I/O architectures may be used as required to perform algorithms. The algorithm and parameter coefficients for the compensated parameters may be changed during instrument operation. If desired, for example in the MoFlo® system, it should be able to be downloaded at operation time from the system's first computer, for example, through the MFIO Rev B Control Word Bus, using the same programming convention.

The additional signal processor(s) used in parallel can provide compensated parameters sufficiently fast that the data from numerous signals, channels, or parameters can have compensation transformation performed simultaneously. The speed of operation on the first

group of 16 data words can occur before the second group of 16 data words becomes available. Each data word can pass through the additional signal processor at a rate of at least every 150 nanoseconds. Consequently, the additional signal processor can perform all operations to which it has been assigned for 16 data words within a maximum period of about 2410 nanoseconds.

As such, compensation transformation operations on the data from a signal(s) can provide compensated parameters to differentiate occurrences during flow cytometer operation. For such real time operation of a flow cytometer or other instrument, the additional signal processor(s) can perform compensation transformation operations for selected parameters even when the occurrences which are being differentiated have a rate of at least 10,000 per second or up to 800,000 occurrences per second. Naturally, the additional signal processor(s) could apply compensation transformation operations to occurrences having lower rates as well.

The compensated parameters generated by the additional signal processor(s) are then returned to the first signal processor. As such, the first signal processor can handle data for different tasks than the additional signal processors. In one embodiment of the invention, the first signal processor can perform the task of data management and display while the additional signal processors are performing, among other others, compensation transformation functions on the original signals. Thus, the separation of the tasks of data management and display and parameter compensation transformation may be an essential requirement to achieve accurate and reliable function.

As but one example of using the invention, with or without additional signal processor(s), compensation transformation, including complex operations, can be performed on the emission spectra of flourescent antibody labels which overlaps the passbands of eight PMT filters. The compensation transformation operations can take the following form, and while this may be a preferred arrangement, a great variety of alternative embodiments are possible.

Two way compensation:

Two linear signals from 0 to 1000 mv converted to a log signal in such a fashion that the log and linear voltages are related:

$$V^1_{log} = A.\log(V^1_{lin}/V_{th}) \quad (1)$$

$$V^2_{log} = A.\log(V^2_{lin}/V_{th}) \quad (2)$$

where

$$A = 10000/\log(10000/V_{th})$$

$V/_{th}$ is normally 1 millivolt. This formula ensures that an input from 1 millivolt to 10000 millivolts will produce a log signal from 0 to 10000 millivolts with 2.5 volts per decade.

A compensated parameter is a parameter with cross-talk subtracted out between two parameters. This is given by:

$$V^{1c}_{lin} = V^1_{lin}(1 - C_{12})\hat{}V^2_{lin}/V^1_{lin} \quad (3)$$

$$V^{2c}_{lin} = V^2_{lin}(1 - C_{21})\hat{}V^1_{lin}/V^2_{lin} \quad (4)$$

In order to recover the V^1_{lin} and V^2_{lin} from the log values, the inverse functions of (1) and (2) may be evaluated:

$$V^1_{lin} = V_{th} \exp(V^1_{log}/A) \quad (5)$$

$$V^2_{lin} = V_{th} \exp(V^2_{log}/A) \quad (6)$$

These linear values may be then applied to (3) and (4) above and converted to log by reapplication of (1) and (2).

In practice, this calculation will be performed on digital values whose linear range is 0 to 4095 (post digitization) and where the threshold value is 4095./10000.0.

Three way compensation.

Mathematically this is the same process except that the formulae for the compensation set is:

$$V^{1c}_{lin} = V^{1}_{lin}(1 - C_{12})\hat{}V^{2}_{lin}/V^{1}_{lin}(1 - C_{13})\hat{}V^{3}_{lin}/V^{1}_{lin} \ldots\ldots \quad (7)$$

$$V^{2c}_{lin} = V^{2}_{lin}(1 - C_{21})\hat{}V^{1}_{lin}/V^{2}_{lin}(1 - C_{23})\hat{}V^{3}_{lin}/V^{2}_{lin} \ldots\ldots \quad (8)$$

$$V^{3c}_{lin} = V^{3}_{lin}(1 - C_{31})\hat{}V^{1}_{lin}/V^{3}_{lin}(1 - C_{32})\hat{}V^{2}_{lin}/V^{3}_{lin} \ldots\ldots \quad (9)$$

and so on for higher order compensation sets.

The lookup tables could be used for N-color compensation in the following way. Following this note on the transformation it is clear that N-color compensation can be deconstructed to N-1 2D lookups. For example, the 3-color compensated output when followed through from anti-log and back to log may look like this:

$$V^{1c}_{log} = V^{1}_{log} - \exp(V^{2}_{log} - V^{1}_{log}).\log(1 - c_{12}) - \exp(V^{3}_{log} - V^{1}_{log}).\log(1-c_{13})$$

Taking the first two terms together and the last term of this expression it is equivalent to:

$$V^{1c}_{log} = LUT(V^{1}log, V^{2}_{log}) - LUT(V^{1}_{log}, V^{3}_{log})$$

Applying the Transformation

The following notation convention is used to describe and eight by eight compensation matrix:

p_{nc} where n = 0 to 7 are the compensated outputs

p_n are the input log signals where n = 0 to 7

c_{jk} are the compensation coefficients = $-A*\log(1-C_{jk})$ where C_{jk} are the fractional compensation values ranging from -.999 to .999

$e(p_n - p_m) = \exp((p_n - p_m)/A)$

A = 4095.0/log (10000) = 444.6

The compensation matrix may be as follows:

$$p_{0c} = p_0-c_{01}.e(p_1-p_0)-c_{02}.e(p_2-p_0)-c_{03}.e(p_3-p_0)-c_{04}.e(p_4-p_0)-c_{06}.e(p_6-p_0)-c_{07}.e(p_7-p_0) \quad (10)$$

$$p_{1c} = -c_{10}.e(p_0-p_1)+p_1-c_{12}.e(p_2-p_1)-c_{13}.e(p_3-p_1)-c_{14}.e(p_4-p_1)-c_{15}e(p_5-p_1)-c_{16}.e(p_6-p_1)-c_{17}.e(p_7-p_1) \quad (11)$$

$$p_{2c} = -c_{20}.e(p_0-p_2)-c_{21}.e(p_1-p_2)+p_2-c_{23}.e(p_3-p_2)-c_{24}.e(p_4-p_2-c_{25}.e(p_5-p_2)-c_{26}.e(p_6-p_2)-c_{27}.e(p_7-p_2) \quad (12)$$

$$p_{3c} = -c_{30}.e(p_0-p_3)-c_{31}.e(p_1-p_3)-c_{32}.e(p_2-p_3)+p_3-c_{34}.e(p_4-p_3)-c_{35}e(p_5-p_3)-c_{36}.e(p_6-p_3)-c_{37}.e(p_7-p_3) \quad (13)$$

$$p_{4c} = -c_{40}.e(p_0-p_4)-c_{41}.e(p_1-p_4)-c_{42}.e(p_2-p_4)-c_{43}.e(p_3-p_4)+p_4-c_{45}e(p_5-p_4)-c_{46}.e(p_6-p_4)-c_{47}.e(p_7-p_4) \quad (14)$$

$$p_{5c} =-c_{50}.e(p_0-p_5)-c_{51}.e(p_1-p_5)-c_{52}.e(p_2-p_5)-c_{53}.e(p_3-p_5)-c_{54}e(p_4-p_5)+p_5-c_{56}.e(p_6-p_5)-c_{57}.e(p_7-p_5) \quad (15)$$

$$p_{6c} = -c_{60}.e(p_0-p_6)-c_{61}.e(p_1-p_6)-c_{62}.e(p_2-p_6)-c_{63}.e(p_3-p_6)-c_{64}e(p_4-p_6)-c_{65}.e(p_5-p_6)+p_6-c_{67}.e(p_7-p_6) \quad (16)$$

$$p_{7c} = -c_{70}.e(p_0-p_7)-c_{71}.e(p_1-p_7)-c_{72}.e(p_2-p_7)-c_{73}.e(p_3-p_7)-c_{74}e(p_4-p_7)-c_{75}.e(p_5-p_7)-c_{76}.e(p_5-p_7)+p_7 \quad (17)$$

Note that the c_{jk} are positive or negative and the parameters from which the others are subtracted are along the diagonal of the matrix.

Properties

- There is symmetry around the diagonal in that the $e(p_j-p_k)$ terms one side of the diagonal are the inverse of those on the other. However this is not a useful symmetry since division is a time consuming operation on an integer arithmetic DSP device.

- The functions $e(p_j-p_k)$ may range from exp(-4095/A) to exp(4095/A) since p_n may be always positive and in the range 0 to 4095. This is a range from 1/10000 to 10000 which is an eight decade range. In order to do fast integer arithmetic, preferably the calculation of $e(p_j-p_k)$ should be done with a 16 bit map to preserve memory space, but the values in the lower ranges less than 1.0 are badly represented. This means that calculation accuracy cannot be maintained across all mapped values of $e(p_k-p_k)$.

- It may be necessary to have two maps, one for positive and the other for negative arguments of e() in order to maintain accuracy.

Given these constraints, we can calculate the number of operations which may be needed to resolve this matrix.

	Operations	Speed (clocks)	Clocks
Pointer Loads	4	4	16
	(2 maps, p_n pointer, c_{jk} pointer		
Sum Initialization	8	1	8
Loads of p_n	8	4	32
Subtracted Pairs	28	1	28

Mappings	56	4	224
Loads of c_{jk}	56	4	224
Multiplies	56	2	112
Subtractions	56	1	56
Stores	8	1	8
Total	280		708

The 6201 DSP runs at a clock cycle of 5 ns. Thus, this calculation for non-optimized execution is 5*708 = 3540 ns. The MoFlo® parameter bus runs at 150 ns per frame word, thus the number of MoFlo® data words is:

$$3540/150 = 23.6$$

The last compensation parameter is in slot 10. The output needs to be ready at data word 16. The calculation matrix cannot be done as each MoFlo® parameter comes across because the off-diagonal elements $e(p_j-p_k)$ may be mixtures of all parameters. The pipelining and parallel architecture of the DSP can allow substantial reduction of this calculation time.

Symmetry reductions can be made on this set in order to reduce execution time. The equations above can be multiplied by $e(p_n)$ and the diagonal terms moved to the left side

$$(p_{0c}-p_0).e(p_o) = 0 \quad -c_{01}.e(p_1)-c_{02}.e(p_2)-c_{03}.e(p_3)-c_{04}.e(p_4)-c_{05}.e(p_5)-c_{06}.e(p_6)-c_{07}.e(p_7)$$

$$(p_{1c}-p_1).e(p_1) = -c_{10}.e(p_0) \quad +0 \quad -c_{12}.e(p_2)-c_{13}.e(p_3)-c_{14}.e(p_4)-c_{15}.e(p_5)-c_{16}.e(p_6)-c_{17}.e(p_7)$$

$$(p_{2c}-p_2).e(p_2) = -c_{20}.e(p_0)-c_{21}.e(p_1) \quad +0 \quad -c_{23}.e(p_3)-c_{24}.e(p_4)-c_{25}.e(p_5)-c_{26}.e(p_6)-c_{27}.e(p_7)$$

$$(p_{3c}-p_3).e(p_3) = -c_{30}.e(p_0)-c_{31}.e(p_1)-c_{32}.e(p_2) \quad +0 \quad -c_{34}.e(p_4)-c_{35}.e(p_5)-c_{36}.e(p_6)-c_{37}.e(p_7)$$

$$(p_{4c}-p_4).e(p_4) = -c_{40}.e(p_0)-c_{41}.e(p_1)-c_{42}.e(p_2)-c_{43}.e(p_3) \quad +0 \quad -c_{45}.e(p_5)-c_{46}.e(p_6)-c_{47}.e(p_7)$$

$$(p_{5c}-p_5).e(p_5) = -c_{50}.e(p_0)-c_{51}.e(p_1)-c_{52}.e(p_2)-c_{53}.e(p_3)-c_{54}.e(p_4) \quad +0 \quad -c_{56}.e(p_6)-c_{57}.e(p_7)$$

$$(p_{6c}-p_6).e(p_6) = -c_{60}.e(p_0)-c_{61}.e(p_1)-c_{62}.e(p_2)-c_{63}.e(p_3)-c_{64}.e(p_4)-c_{65}.e(p_5) \quad +0 \quad -c_{67}.e(p_7)$$

$$(p_{7c}-p_7).e(p_7) = -c_{70}.e(p_0)-c_{71}.e(p_1)-c_{72}.e(p_2)-c_{73}.e(p_3)-c_{74}.e(p_4)-c_{75}.e(p_5)-c_{76}.e(p_6) \quad +0$$

	Operation	Speed (clocks)	Clocks
Pointer loads	4	2	8
Sum initialization	8	1	8
Loads of pn	8	4	32
Mappings	8	4	32
Loads of c_{jk}	32	4	128
Multiplies	64	2	128
Subtractions	64	1	64
Post NORM	8	1	8
Post SHL	8	1	8
Pointer loads	2	2	4
Post loads	8	4	32
Post remap	8	4	32
Post multiples	8	2	16
Post SHIFT ADD	8	1	8
Post SHR	8	1	8
Post adds	8	1	8
Stores	8	1	8
Total			532

The execution time for this matrix is 2660 ns which is MoFlo® frame words = 2660/150 = 17.7 MoFlo® data words.

Using the linear assembler and optimization of register usage to enhanced parallel operation can yield the parallel code set out in Attachment A, hereby incorporated by reference herein. This program, and the above-described example is not meant to limit the invention to specific hardware, software, algorithms, applications, or arrangements, but is

provided as a guide in making and using the invention which may take the form of various embodiments. Particular embodiments of the invention, in the flow cytometry context or otherwise, can be as follows.

In certain applications, occurrences can be separated in time. Occurrences separated in time can be, in the flow cytometer context, for example, different original or raw signals generated for the same particle as it moves through the various flow cytometer processes which as above-described involve entrainment into a fluid stream, excitation of bound fluorochrome, assignment to a class, and separation of particles to the assigned classes. Occurrences separated in time can also involve a particle labeled with several different fluorochromes with each type of fluorochrome excited at different points in time. Again occurrences separated in time, could be a series of discrete occurrences each monitored for the same parameter, such as a fluorescent emission from a series of labeled cells, or it could be a single occurrence monitored at discrete periods in time, such as the characteristics of a flourescent emission as it decays. Of course, numerous other examples could be provided which have occurrences separated in time. The spatial separation of these occurrences leads to original signal output which is separated in time. The use of additional signal processor(s) using pass through, compensation transformation, and return can remove this temporal separation. In some cases this will enable certain application which were heretofore not possible, such as the use of multiple separate lasers to excite multiple fluorochromes over time, in other cases it will allow the original signals to have compensation transformation applied and more accurate differentiations made between occurrences even during operation of the instrument. Operations such as this which are have a low tolerance for "time jitter" often cannot be performed using an analog arrangement because of the difficulty of removing the temporal separation with analog circuitry.

In certain applications "cross talk" between the same or different parameters can occur. Compensation transformation on the original or raw signals can remove "cross-talk" between the same or different parameters which are incident to the same or different occurrences. different occurrences incident to the same parameters, or "cross talk" incident to. As described above, the "cross talk" between different types of fluorochrome emission was compensated. Compensation transformation may allow the raw original fluorescent signals, or numerous

other types of signals, to be compensated so that the resulting compensated parameter has the cross-talk accurately removed and blank reference signals correctly positioned. This may be particularly relevant to other types of applications such as the detection of defects in products as disclosed by United States Patent No. 4, 074, 809 and 4,501,366; field flow fractionation, liquid chromatography, or electrophoresis as disclosed by United States Patent No. 5,503,994; computer tomography, gamma cameras, or time of flight instruments as disclosed by United States Patent No. 5,880, 457; or flow cytometry as disclosed by United States Patent 5,135,759. with respect to bright fluorescent values, or as described by United States Patent Application No. 60/2103089, each hereby incorporated by reference herein. This type of compensation transformation can be performed on numerous channels simultaneously, at least 8 channels in the above-described example, and provides orthogonalized data which can be returned to the first signal processor.

Certain applications require multiple color compensation. Compensation transformation for multiple color compensation can take the format presented above and allow for at least 8 color compensation embodied by a 64 element matrix of operations. The transformation can operate on linear or logarithmic format data. Naturally, as explained higher order set can be used providing for N-color compensation.

Certain application require analysis of parameter kinetics or ratios. Ratios between two signals over time can be an important measurement in the study of cell kinetics. The original signals can be compensated such that the ratio can be used to provide a measure of absolute differences between the signals. For example, calcium release can be an important measurement for the study of cell kinetics. A ratio of two fluorescent emission signals can be required to provide a measure of calcium release. These fluorescent emission signals can have compensation transformation applied to provide compensated fluorescent emission signals for comparison in the appropriate time frame required to maintain accuracy. Multiple ratios can also be performed. Time can also be a parameter essential for kinetic measurements and can be supplied by the on-board clock. The on-board clock can have a time range from microseconds to years allowing full flexibility in time-stamping data streams.

Certain applications require differentiation of and tracking of sub-populations. Flow cytometers depend on the stability of various parameters, including, but not limited to, environmental parameters, instrument parameters, occurrence parameters to analyze and define the mean and width of particle populations. Unfortunately, these parameters can be in continuous dynamic instability. Stability can be controlled by compensation transformation of the original signals from these various parameters. Alternately, compensation transformation can track the drift in these parameters. Compensation transformation of original signal information can allow for the selection of parameters to resolve or differentiate sub-population, to select the level of resolution to be maintained between individuals of sub-populations, to select the thresholds for assignment and separation of individuals from sub-populations, to allow for continuous differentiation and assignment of individuals from sub-populations to various classes, to track sub-populations as parameters drift, to assess the purity of pools of separated individuals without re-analysis, among other applications.

In this regard, two dimensional, three dimensional, or higher dimensional populations of particles can be differentiated and assigned to various sub-populations and multi-dimension regions can be used to separate the sub-populations when using the invention. This provides a powerful and direct method of multi-dimensional sub-population separation that has been previously unavailable on flow cytometers, and on other types of instrument, and in other fields of application .

Another aspect, of sub-population identification involves closely overlapping sub-populations can be enumerated by dynamically characterizing the overlap using compensation transformations that may be designed to detect the proportion of overlaps. The exact proportions, mean, width and separation of multi-featured sub-populations can also be characterized with the invention. Extensive populations of particles with small sub-populations of interest can be focused upon and held in dynamic amplification or focus through transformation compensation of amplification parameters such that the sub-populations of interest can be defined, located, analyzed, and separated. Without transformation compensation, such accurate delineation may not be possible.

In applications using flow cytometry, particles with various population(s)/sub-populations of interest can be screened and regions of interest can be created which delineate these populations. These regions can be automatically assigned to the sorting electronics of a flow cytometer so that real-time physical separation of the particles of interest can be sorted. This automation process can be important when flow cytometry is used to separate high volumes of certain types of cells for culturing, transfecting, insemination, biochemical recombination, protein expression, or the like.

Populations of particles can be stored in the memory of the addition signal processor(s) using binning transformations. The statistical characterization of these populations, such as mean, standard deviation, skewness and separation can be returned to the first signal processor, that can be a workstation for display, storage, or retrieval of data. Thus off-loading this task to the additional signal processor can increase the performance of the workstation.

The method described above and detailed in Attachment A can preserve the raw signal data in a memory storage element. Cost considerations often exclude this feature on an analog systems. Saving raw or original signal data also conforms to Good Manufacturing Practice in that the original signal data can be retrieved if the transformed data has been incorrectly manipulated. By saving the original signal data and duplicating original signal data for further processing, elements of the original raw signal data that may be lost by digital 'roofing' or 'flooring' can be maintained. This can allow original signal retrieval and data backtracking for FDA requirements and for signal re-analysis.

Now referring to Figure 2, a preferred embodiment of the hardware with respect to an application of the invention with the MoFlo® flow cytometer is shown. As can be understood, the additional signal processor (17) can be located internal to or external to the core of the instrument. A minimum data memory size of 56 kilowords of 12 bits or wider may be required for each compensation transformation operation (based on the example above). A minimum I/O memory space of TBD kilowords may also be required. Various CPLD/FPGA or digital signal processing Von Neuman and Harvard program, data, and I/O architectures,

or the like, may be used to perform compensation transformation algorithms, such as those specified above.

Additional processors (17) serve to increase the parallelism of the operations, thus allowing transformations at hitherto unachievable speeds. This increased power allows operations that are algebraic as well as approximately transcendental. Transcendental operations can be considered those requiring an infinite number of steps. However extremely high processing rates can provide approximations to the infinite that are practicable and indistinguishable from an exact computation.

As can be easily understood from the foregoing, the basic concepts of the present invention may be embodied in a variety of ways. It involves both signal processing techniques as well as devices to accomplish the appropriate signal processing. In this application, the processing techniques are disclosed as part of the results shown to be achieved by the various devices described and as steps which are inherent to utilization. They are simply the natural result of utilizing the devices as intended and described. In addition, while some devices are disclosed, it should be understood that these not only accomplish certain methods but also can be varied in a number of ways. Importantly, as to all of the foregoing, all of these facets should be understood to be encompassed by this disclosure.

The discussion included in this provisional application is intended to serve as a basic description. The reader should be aware that the specific discussion may not explicitly describe all embodiments possible; many alternatives are implicit. It also may not fully explain the generic nature of the invention and may not explicitly show how each feature or element can actually be representative of a broader function or of a great variety of alternative or equivalent elements. Again, these are implicitly included in this disclosure. Where the invention is described in functionally-oriented terminology, each aspect of the function is accomplished by a device, subroutine, or program. Apparatus claims may not only be included for the devices described, but also method or process claims may be included to address the functions the invention and each element performs. Neither the description nor the terminology is intended to limit the scope of the claims which now be included.

Further, each of the various elements of the invention and claims may also be achieved in a variety of manners. This disclosure should be understood to encompass each such variation, be it a variation of an embodiment of any apparatus embodiment, a method or process embodiment, or even merely a variation of any element of these. Particularly, it should be understood that as the disclosure relates to elements of the invention, the words for each element may be expressed by equivalent apparatus terms or method terms -- even if only the function or result is the same. Such equivalent, broader, or even more generic terms should be considered to be encompassed in the description of each element or action. Such terms can be substituted where desired to make explicit the implicitly broad coverage to which this invention is entitled. As but one example, it should be understood that all actions may be expressed as a means for taking that action or as an element which causes that action. Similarly, each physical element disclosed should be understood to encompass a disclosure of the action which that physical element facilitates. Regarding this last aspect, as but one example, the disclosure of a "processor" should be understood to encompass disclosure of the act of "processing" -- whether explicitly discussed or not -- and, conversely, were there only disclosure of the act of "processing", such a disclosure should be understood to encompass disclosure of a "processor" and even a means for "processing". Such changes and alternative terms are to be understood to be explicitly included in the description.

Additionally, the various combinations and permutations of all elements or applications can be created and presented. All can be done to optimize the design or performance in a specific application.

Any acts of law, statutes, regulations, or rules mentioned in this application for patent: or patents, publications, or other references mentioned in this application for patent are hereby incorporated by reference. Specifically, United States Patent Application No. 60/160,719 is hereby incorporated by reference herein including any figures or attachments, and each of references in the following table of references are hereby incorporated by referencece.

I. U.S. PATENT DOCUMENTS

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In addition, as to each term used it should be understood that unless its utilization in this application is inconsistent with such interpretation, common dictionary definitions should be understood as incorporated for each term and all definitions, alternative terms, and synonyms such as contained in the Random House Webster's Unabridged Dictionary, second edition are hereby incorporated by reference. However, as to each of the above, to the extent that such information or statements incorporated by reference might be considered inconsistent with the patenting of this/these invention(s) such statements are expressly not to be considered as made by the applicant(s).

In addition, unless the context requires otherwise, it should be understood that the term "comprise" or variations such as "comprises" or "comprising", are intended to imply the inclusion of a stated element or step or group of elements or steps but not the exclusion of any other element or step or group of elements or steps. Such terms should be interpreted in their most expansive form so as to afford the applicant the broadest coverage legally permissible in countries such as Australia and the like.

Thus, the applicant(s) should be understood to have support to claim at least: i) each of the processing devices or subroutines as herein disclosed and described, ii) the related methods disclosed and described, iii) similar, equivalent, and even implicit variations of each

of these devices and methods, iv) those alternative designs which accomplish each of the functions shown as are disclosed and described, v) those alternative designs and methods which accomplish each of the functions shown as are implicit to accomplish that which is disclosed and described, vi) each feature, component, and step shown as separate and independent inventions, vii) the applications enhanced by the various systems or components disclosed, viii) the resulting products produced by such systems or components, ix) methods and apparatuses substantially as described hereinbefore and with reference to any of the accompanying examples, x) the various combinations and permutations of each of the elements disclosed, xi) processes performed with the aid of or on a computer as described throughout the above discussion, xii) a programmable apparatus as described throughout the above discussion, xiii) a digitally readable memory encoded with data to direct a processor comprising means or elements which function as described throughout the above discussion, xiv) a computer configured as herein disclosed and described, xv) individual or combined subroutines and programs as herein disclosed and described, xvi) the related methods disclosed and described, xvii) similar, equivalent, and even implicit variations of each of these systems and methods, xviii) those alternative designs which accomplish each of the functions shown as are disclosed and described, xix) those alternative designs and methods which accomplish each of the functions shown as are implicit to accomplish that which is disclosed and described, xx) each programmable feature, component, and step shown as separate and independent inventions, and xxi) the various combinations and permutations of each of the above.

VI. CLAIMS

1. A method of flow cytometry, comprising the steps of :

a. establishing a fluid stream;

b. entraining particles in said fluid stream;

c. perturbing said fluid stream;

d. sensing a first occurrence incident to at least one particle;

e. generating a first signal;

f. producing data from said first signal;

g. sensing at least one additional occurrence incident to said at least one particle;

h. generating at least one additional signal;

i. producing data from said at least one additional signal;

j. processing data from said first signal;

k processing data from said at least one additional signal;

l. applying at least one transformation operation to processed data from said first signal;

m. applying at least one transformation operation to processed data from said at least one additional signal;

n. compensating at least one parameter shared by said first occurrence and said at least one additional occurrence;

o. differentiating said first occurrence from said at least one additional occurrence based upon at least one compensated parameter;

p. assigning said at least one particle to a class;

q. separating assigned particles; and

r. collecting said assigned particles by class.

2. A method of flow cytometry as described in claim 1, wherein said steps of sensing a first occurrence incident to at least one particle and sensing at least one additional occurrence incident to said at least one particle comprise sensing occurrences incident to a single particle.

3. A method of flow cytometry as described in claim 1, wherein said steps of sensing a first occurrence incident to at least one particle and sensing at least one additional

occurrence incident to said at least one particle comprise sensing occurrences incident to at least two particles.

4. A method of flow cytometry as described in claim 2, wherein said step of sensing occurrences incident to a single particle comprise sensing serial occurrences.

5. A method of flow cytometry as described in claim 3, wherein said step of sensing occurrences incident to at least two particles comprise sensing serial occurrences.

6. A method of flow cytometry as described in claim 4, wherein said step of sensing occurrences incident to a single particle comprise sensing parallel occurrences.

7. A method of flow cytometry as described in claim 5, wherein said step of sensing occurrences incident to at least two particles comprise sensing parallel occurrences.

8. A method of flow cytometry as described in claim 8, wherein said step of sensing occurrences incident to a single particle comprise sensing occurrences incident to the same parameter.

9. A method of flow cytometry as described in claim 2, wherein said step of sensing occurrences incident to a single particle comprises sensing occurrences incident to at least two parameters.

10. A method of flow cytometry as described in claim 3, wherein said step of sensing occurrences incident to at least two particles comprise sensing occurrences incident to the same parameter.

11. A method of flow cytometry as described in claim 3, wherein said step of sensing occurrences incident to at least two particles comprise sensing occurrences incident to at least two parameters.

12. A method of flow cytometry as described in claim 1, wherein said steps of producing data from said first signal and generating at least one additional signal comprise generating a single channel of information.

13. A method of flow cytometry as described in claim 1, wherein said steps of producing data from said first signal and generating at least one additional signal comprise generating multiple channels of information.

14. A method of flow cytometry as described in claim 1, wherein said steps of producing data from said first signal and generating at least one additional signal comprises generating signals at a rate of at least 10,000 per second.

15. A method of flow cytometry as described in claim 1, wherein said step of separating said differentiated component from said substance comprises:

a. encapsulating said differentiated component in a droplet; and

b. collecting droplets having differentiated components of a class into a container.

16. A method of flow cytometry as described in claim 15, wherein said step of collecting droplets having differentiated components of a class into a container occurs at a rate of at least 1000 per second.

17. A method of flow cytometry as described in claim 1, wherein said step of applying compensation information to processed data from said first signal and to processed data from said at least one additional signal comprising the step of performing complex operations on said processed data from first signal and to processed data from said second signal.

18. A method of flow cytometry as described in claim 17, wherein said step of performing complex operations on said processed data from first signal and to processed data from said second signal comprises performing algebraic operations.

19. A method of flow cytometry as described in claim 18, wherein said step of performing algebraic operations comprises:

a. applying a parameter compensation transformation to said first signal and to said at least one additional signal;

b. generating a first compensated signal and at least one additional compensated signal; and

c. comparing said first compensated signal and said at least one additional compensated signal;

20. A method of flow cytometry as described in claim 19, wherein said step of comparing said first compensated signal and said at least one additional compensated signal comprises minimizing characteristics shared by a parameter.

21. A method of flow cytometry as described in claim 19, wherein said step of comparing said first compensated signal and said at least one additional compensated signal comprises minimizing characteristics shared by said at least two different parameters.

22. A method of flow cytometry as described in claim 20, wherein said step of minimizing characteristics shared by said same parameter comprises reducing spectrum overlap.

23. A method of flow cytometry as described in claim 21, wherein said step of minimizing characteristics shared by said at least two different parameters comprises reducing spectrum overlap.

24. A method of flow cytometry as described in claim 22, wherein said step of minimizing characteristics shared said one parameter comprises substantially aligning temporal serial events.

25. A method of flow cytometry as described in claim 23, wherein said step of minimizing characteristics shared by said at least two different parameters comprises substantially aligning temporal serial events.

26. A method of flow cytometry as described in claim 1, further comprising the step of processing said data from said first signal and said data from said one additional signal using at least one additional signal processor.

27. A method of flow cytometry as described in claim 26, wherein said step of using at least one additional signal processor comprises using at least one additional signal processor in parallel with said first signal processor.

28. A method of flow cytometry as described in claim 27, wherein said step of using at least one additional signal processor in parallel with said first signal processor comprises using said first signal processor and said second signal processor simultaneously.

29. A method of flow cytometry as described in claim 28, wherein said step of using said first signal processor and said second signal processor simultaneously comprises registering usage of a parallel linear code.

30. A method of flow cytometry as described in claim 29, wherein said step of registering usage of a parallel linear code comprises registering digital parallel linear code.

31. A method of flow cytometry as described in claim 30, further comprising the step of applying a compensation matrix.

32. A method of flow cytometry as described in claim 31, further comprising the step of reducing execution time using compensation matrix symmetry reductions.

33. A method of flow cytometry as described in claim 1, further comprising the steps of:

a. assaying original data from said first signal and said at least one additional signal in a memory storage element; and

b. retrieving original data from said first signal and said at least one additional signal saved in said memory storage element without alteration.

34 A method of flow cytometry as described in claim 33, further comprising the steps of:

a. duplicating said original data from said first signal and said at least one additional signal;

b. processing a duplicate signal;

c. interpreting said first occurrence and said at least one additional occurrence using a processed duplicate signal.

35. A method of flow cytometry as described in claim 34, further comprising the step of binning information in said at least one additional signal processor.

36. A flow cytometer comprising:

a. a fluid stream;

b. at least one particle entrained in said fluid stream;

c. an oscillator;

d. a first sensor;

e. at least one signal generator;

f. data from said signal generator incident to a first occurrence;

g. data from from said signal genrator incident to at least one additional occurrence;

h. a signal processor;

i. a transformation operation applied to at least a portion of said data from said signal generator incident to said first occurrence;

j. a transformation operation applied to at least a portion of said data from said signal generator incident to said second occurrence;

k. a compensated parameter shared by said said first occurrence and by said second occurrence;

l. a particle differentiation element;

n. a particle assignment element;

o. a particle separator; and

p. at least one container in which separated particles are collected.

37. A flow cytometer as described in claim 36, wherein said signal processor performs complex transformation operations to said at least a portion of said data from said signal generator incident to said first occurrence and to at least a portion of said data from said signal generator incident to said second occurrence.

38. A flow cytometer as described in claim 37, further comprising at least one additional signal processor.

39. A flow cytometer as described in claim 38, wherein said at least one additional signal processor performs said complex transformation operations to said at least a portion of said data from said signal generator incident to said first occurrence and to at least a portion of said data from said signal generator incident to said second occurrence.

40. A flow cytometer as described in claim 39, wherein said at least one additional signal processor is a digital signal processor.

41. A flow cytometer as described in claiM 40, further comprising a memory element responsive to said digital signal processor, wherein original data from said signal generators can be stored.

42. A flow cytometer as described in claim 41, further comprising an original data retreival element.

43. A flow cytometer as described in claim 42, further comprising an original data duplication element.

44. A flow cytometer as described in claim 43, further comprising a binning element.

45.. A method of flow cytometry, comprising the steps of:

a. establishing a fluid stream;

b. perturbing said fluid stream;

c. sensing an occurrence incident to said fluid stream;

d. generating a signal from said occurrence;

e. processing said signal using a first signal processor;

f. processing said signal using at least one additional signal processor; and

g. combining output from said first signal processor and said at least one additional signal processor;

h. applying combined output to classify said occurrence.

46. A method of flow cytometry as described in claim 45, wherein said step of processing said signal using at least one additional signal processor comprises using at least one additional signal processor in parallel with said first signal processor.

47. A method of flow cytometry as described in claim 46, wherein said step of using at least one additional signal processor in parallel with said first signal processor comprises processing at least a portion of said signal using said first signal processor and said second signal processor simultaneously.

48. A method of flow cytometry as described in claim 47, wherein said step of processing at least a portion of said signal using said first signal processor and said second signal processor simultaneously comprises registering usage of a parallel linear code.

49. A method of flow cytometry as described in claim 48, wherein said step of registering usage of a parallel linear code comprises registering usage of a parallel digitized code.

50. A method of flow cytometry as described in claim 49, further comprises the steps of:

a. performing compensation transformation on said signal; and

b. generating a compensated signal.

51. A method of flow cytometry as described in claim 50, wherein said step of performing compensation transformation on said signal comprises compensating a single parameter.

52. A method of flow cytometry as described in claim 51, wherein said step of compensating a single parameter comprises compensating an analog signal.

53. A method of flow cytometry as described in claim 50, wherein said step of compensating an analog signal comprises minimizing variations selected from the group consisting of phase, or shape.

54. A method of flow cytometry as described in claim 50, wherein said step of performing compensation transformation on said signal comprises compensating at least two different parameters.

55. A method of flow cytometry as described in claim 54, wherein said step of compensating at least two different parameters comprises minimizing characteristics shared by said at least two different parameters.

56. A method of flow cytometry as described in claim 55, wherein said step of minimizing characteristics shared by said at least two different parameters comprises reducing spectrum overlap.

57. A method of flow cytometry as described in claim 50, wherein said step of performing compensation transformation comprises applying algebraic operations.

58. A method of flow cytometry as described in claim 57, further comprises applying a compensation matrix.

59. A method of flow cytometry as described in claim 58, further comprises the step of minimizing execution time of performing compensation transformation on said signal by utilizing symmetry reductions in said compensation matrix.

60. A method of flow cytometry as described in claim 45, wherein said step of performing compensation transformation on said signal comprises performing compensation transformation on signals generated from at least 10,0000 occurrences per second.

61. A method of flow cytometry as described in claim 45, further comprising the step of binning information in said at least one additional signal processor.

62. A method of flow cytometry as described in claim 45, further comprising the steps of:
    a. assaying original data from said signal in a memory storage element; and
    b. retrieving original data from said signal saved in said memory storage element without alteration.

63. A method of flow cytometry as described in claim 45, further comprising the steps of:
    a. duplicating said original data from said signal;
    b. processing a duplicate signal; and
    c. interpreting said occurrence using a processed duplicate signal.

64. A flow cytometer comprising:
    a. a fluid stream;
    b. a sensor responsive to an occurrence;
    c. at least one signal generator coupled to said sensor;
    d. a first signal processor to perform operations on signal data;
    e. a second signal processor to perform operations on said signal data;
    f. compensated parameter output from said second signal processor returned to said first signal processor; and
    g. a particle differentiation element resposive to said compensated parameter output.

65. A flow cytometer as described in 64, further comprising
    a. a particle assignment element;
    b. a particle separator; and
    c. at least one container in which separated particles are collected.

66. A method of flow cytometry, comprising the steps of:
    a. establishing a fluid stream;
    b. generating a first signal incident to said fluid stream;

c. generating at least one additional signal incident to said fluid stream;

d. applying a parameter compensation transformation to said first signal and to said at least one additional signal;

e generating a first compensated signal and at least one additional compensated signal;

f. comparing said first compensated signal and said at least one additional compensated signal;

g. classifying occurrences based upon comparison of said first compensated signal and said at least one additional compensated signal.

67. A method of flow cytometry as described in 66, further comprising the step of applying a compensation matrix on output from said parameter compensation transformation.

68. A method of flow cytometry as described in 67, further comprising the step of using symmetry reductions within said compensation matrix to reduce execution time.

69. A method of flow cytometry as described in 68, further comprising the step of digitizing said first signal and said at least one additional signal.

70. A method of flow cytometry as described in 69, further comprising the step of processing said first signal and said at least one additional signal using at least one additional signal processor in parallel with a first signal processor.

71. A method of flow cytometry as described in claim 70, wherein said step of using at least one additional signal processor in parallel with said first signal processor comprises processing at least a portion of said signal using said first signal processor and said second signal processor simultaneously.

72. A method of flow cytometry as described in claim 71, wherein said step of processing at least a portion of said signal using said first signal processor and said second signal processor simultaneously comprises registering usage of a parallel digital linear code.

73. A method of flow cytometry as described in any ne of claims 66, 67, 68, 69, 70, 71, or 72, wherein said step of performing compensation transformation on said first signal and at least one additional signal comprises performing compensation transformation at a rate of least 10,0000 transformations per second.

74. A method of flow cytometry as described in claim 73, further comprising the step of binning information in said at least one additional signal processor.

75. A method of flow cytometry as described in claim 74, further comprising the steps of:
    a. assaying original data from said first signal and said at least one additional signal in a memory storage element; and
    b. retrieving original data from said first signal and said at least one additional signal saved in said memory storage element without alteration.

76. A method of flow cytometry as described in claim 75, further comprising the steps of:
    a. duplicating said original data from said first signal and said at least one additional signal;
    b. processing a duplicate signal; and
    c. interpreting said occurrence using a processed duplicate signal.

77. A flow cytometer comprising:
    a. a fluid stream;
    b. a sensor responsive to an occurrence;
    c. at least one signal generator coupled to said sensor;
    d. a first signal generated by said at least one signal generator;
    e. at least one additional signal generated by said at least one signal generator;
    f. a signal processor responsive to said first signal and to said at least one additional signal;
    g. a transformation operation applied to said first signal and to said at least one additional signal;
    h. a compensated signal comparison element; and

i. a particle differentiation element resposive to said compensated signal comparison element.

78. A method of flow cytometry, comprising the steps of :

a. establishing a fluid stream;

b. perturbing said fluid stream;

c. sensing an occurrence incident to said fluid stream;

d. generating a signal from said occurrence;

e. performing complex operations on said signal; and

f. applying output from said complex operations to perform flow cytometry.

79. A method of flow cytometry as described in claim 78, wherein said step of performing complex operations on said signal comprises performing algebraic operations on said signal.

80. A method of flow cytometry as described in claim 79, wherein said step of performing algebraic operations on said signal comprises performing compensation transformation on said signal.

81. A method of flow cytometry as described in claim 80, wherein said step of performing compensation transformation on said signal comprises compensating a single parameter.

82. A method of flow cytometry as described in claim 81, wherein said step of compensating a single parameter comprises compensating a serial analog signal.

83 A method of flow cytometry as described in claim 80, wherein said step of performing compensation transformation on said signal comprises compensating at least two parameters.

84. A method of flow cytometry as described in claim 83, wherein said step of compensating at least two parameters comprises compensating at least two serial analog signals.

85. A method of flow cytometry as described in claims 81 or 84, wherein said step of compensating said serial analog signals comprises minimizing variations selected from the group consisting of phase or shape.

86. A method of flow cytometry as described in claim 80, wherein said step of performing compensation transformation comprises minimizing shared characteristics.

87. A method of flow cytometry as described in claim 80, wherein said step of minimizing shared characteristics comprises reducing spectrum overlap.

88. A method of flow cytometry as described in claim 80, wherein said step of performing compensation transformation comprises substantially aligning temporal serial events.

89. A method of flow cytometry as described in claim 80, further comprising the step of applying a compensation matrix.

90. A method of flow cytometry as described in claim 89, further comprising the step of reducing execution time using compensation matrix symmetry reductions.

91. A method of flow cytometry as described in claim 78, further comprising the step of processing said data from said signal using at least one additional signal processor.

92. A method of flow cytometry as described in claim 91, wherein said step of using at least one additional signal processor comprises using at least one additional signal processor in parallel with a first signal processor.

93. A method of flow cytometry as described in claim 92, wherein said step of using at least one additional signal processor in parallel with said first signal processor

comprises using said first signal processor and said second signal processor simultaneously.

94. A method of flow cytometry as described in claim 93, wherein said step of using said first signal processor and said second signal processor simultaneously comprises registering usage of a parallel linear code.

95. A method of flow cytometry as described in claim 94, wherein said step of registering usage of a parallel linear code comprises registering digital parallel linear code.

96. A method of flow cytometry as described in claim 91, further comprising the steps of:
   a. assaying original data from said first signal and said at least one additional signal in a memory storage element; and
   b. retrieving original data from said first signal and said at least one additional signal saved in said memory storage element without alteration.

97. A method of flow cytometry as described in claim 96, further comprising the steps of:
   a. duplicating said original data from said first signal and said at least one additional signal;
   b. processing a duplicate signal;
   c. interpreting said first occurrence and said at least one additional occurrence using a processed duplicate signal.

98. A method of flow cytometry as described in claim 97, further comprising the step of binning information in said at least one additional signal processor.

99. A flow cytometer comprising:
   a. a fluid stream;
   b. a sensor responsive to an occurrence;
   c. at least one signal generator coupled to said sensor;
   d. a signal generated by said at least one signal generator;

e. a signal processor responsive to said first signal and to said at least one additional signal;

f. a complex operation applied to said signal; and

g. a compensated signal.

100. A method of flow cytometry, comprising the steps of:

a. establishing a fluid stream;

b. entraining a substance in said fluid stream;

c. perturbing said fluid stream;

d. sensing an occurrence incident to said substance;

e. generating a signal from said occurrence;

f. digitizing said signal; and

g. interpreting said occurrence using said digitized signal.

101. A method of flow cytometry as described in claim 100, further comprising the step of sensing serial occurrences.

102. A method of flow cytometry as described in claim 100, further comprising the step of sensing multiple parallel serial occurrences.

103. A method of flow cytometry as described in claims 101 or 102, wherein said serial occurrences have a rate of at least 10,000 per second.

104. A method of flow cytometry as described in claim 103, further comprising the step of saving said digitized signal in a memory storage element.

105. A method of flow cytometry as described in claim 104, further comprising the step of duplicating said digitized signal saved in said memory storage element.

106. A method of flow cytometry as described in claim 105, further comprising the step of processing a duplicate digitized signal using a first signal processor.

107. A method of flow cytometry as described in claim 106, further comprising the step of processing said duplicate digitized signal using at least one additional signal processor.

108. A method of flow cytometry as described in claim 107, wherein said step of using at least one additional signal processor in parallel with said first signal processor comprises processing at least a portion of said duplicate digitized signal using said first signal processor and said second signal processor simultaneously.

109. A method of flow cytometry as described in claim 108, wherein said step of processing at least a portion of said digitized signal using said first signal processor and said second signal processor simultaneously comprises registering usage of a parallel digitized linear code.

110. A method of flow cytometry as described in claim 109, further comprising the step of performing complex operations on said digital signal.

111. A method of flow cytometry as described in claim 110, wherein said step of performing complex operations on said digital signal comprises performing algebraic operations on said digitized signal.

112. A method of flow cytometry as described in claim 111, wherein said step performing algebraic operations on said digitized signal comprises performing compensation transformation on said signal.

113. A method of flow cytometry as described in claim 112, wherein said step of performing compensation transformation on said signal comprises compensating a single parameter.

114. A method of flow cytometry as described in claim 113, wherein said step of compensating a single parameter comprises compensating a serial analog signal.

115. A method of flow cytometry as described in claim 114, wherein said step of compensating said serial analog signal comprises minimizing variations selected from the group consisting of phase, or shape.

116. A method of flow cytometry as described in claim 112, wherein said step of performing compensation transformation on said signal comprises compensating at least two different parameters.

117. A method of flow cytometry as described in claim 116, wherein said step of compensating at least two different parameters comprises minimizing characteristics shared by said at least two different parameters.

118. A method of flow cytometry as described in claim 117, wherein said step of minimizing characteristics shared by said at least two different parameters comprises reducing spectrum overlap.

119. A method of flow cytometry as described in claim 118, further comprising the step of generating a compensated signal.

120. A method of flow cytometry as described in claim 119, further comprising the step of re-processing said digitized signal.

121. A method of flow cytometry as described in claim 120, wherein said step of interpreting said occurrence using said digitized signal comprises using said compensated signal to differentiate components of said substance.

122. A method of flow cytometry as described in claim 121, further comprising the step of classifying a differentiated component.

123. A method of flow cytometry as described in claim 122, further comprising the step of separating said differentiated component from said substance.

124. A method of flow cytometry as described in claim 123, wherein said step of separating said differentiated component from said substance comprises:

a. encapsulating said differentiated component in a droplet; and

b. collecting droplets having differentiated components of a class into a container.

125. A method of flow cytometry as described in claim 124, wherein said step of collecting droplets having differentiated components of a class into a container occurs at a rate of at least 1000 per second.

126. A flow cytometer, comprising:

a. a fluid stream;

b. a substance entrained in said fluid stream;

c. a sensor responsive to an occurrence incident to said substance entrained in said fluid stream;

d. at least one signal generator coupled to said sensor;

e. signal data generated by said at least one signal generator;

f. a signal data digitizer; and

g. a digital signal processor responsive to a digitized signal.

127. A method of flow cytometry, comprising the steps of:

a. establishing a fluid stream;

b. sensing an occurrence incident to said fluid stream;

c. generating an original signal from said occurrence;

d. saving said original signal in a memory storage element;

e. duplicating said original signal;

f. processing a duplicate signal;

g. interpreting said occurrence using a processed duplicate signal.

128. A method of flow cytometry as described in claim 127, further comprising the step of retrieving said original signal saved in said memory storage element without alteration.

129. A method of flow cytometry as described in claim 128, further comprising the step of re-processing said duplicate signal.

130. A method of flow cytometry as described in claim 129, further comprising the step of re-processing said retrieved original signal.

131. A method of flow cytometry as described in claim 130, further comprising the step of entraining a substance in said fluid stream.

132. A method of flow cytometry as described in claim 131, wherein said step of interpreting said occurrence using said processed duplicate signal comprises using said processed duplicate signal to differentiate components of said substance.

133. A method of flow cytometry as described in claim 132, further comprising the step of classifying a differentiated component.

134. A method of flow cytometry as described in claim 133, further comprising the step of separating said differentiated component from said substance.

135. A method of flow cytometry as described in claim 134, wherein said step of separating said differentiated component from said substance comprises:

a. encapsulating said differentiated component in a droplet; and

b. collecting droplets having differentiated components of a class into a container.

136. A method of flow cytometry as described in claim 135, wherein said step of collecting droplets having differentiated components of a class into a container occurs at a rate of at least 1000 per second.

137. A method of flow cytometry as described in claim 127, wherein said step of sensing an occurrence incident to said fluid stream comprises sensing serial occurrences.

138. A method of flow cytometry as described in claim 127, wherein said step of sensing an occurrence incident to said fluid stream comprises sensing multiple parallel serial occurrences.

139. A method of flow cytometry as described in claim 138, wherein said serial occurrences have a rate of at least 10,000 per second.

140. A method of flow cytometry as described in claim 127, further comprising the step of digitizing said original signal from said occurrence.

141. A method of flow cytometry as described in claim 140, further comprising the step of processing said duplicate digitized signal using at least one additional signal processor.

142. A method of flow cytometry as described in claim 141, wherein said step of using at least one additional signal processor in parallel with said first signal processor comprises processing at least a portion of said duplicate digitized signal using said first signal processor and said at least one additional signal processor simultaneously.

143. A method of flow cytometry as described in claim 142, wherein said step of processing at least a portion of said duplicate digitized signal using said first signal processor and said at least one additional signal processor simultaneously comprises registering usage of a parallel digitized linear code.

144. A method of flow cytometry as described in claim 141, further comprising the step of performing complex operations on said duplicate digitized signal.

145. A method of flow cytometry as described in claim 144, wherein said step of performing complex operations on said duplicate digitized signal comprises performing algebraic operations on said duplicate digitized signal.

146. A method of flow cytometry as described in claim 145, wherein said step performing algebraic operations on said duplicate digitized signal comprises performing compensation transformation on said duplicate digitized signal.

147. A method of flow cytometry as described in claim 146, wherein said step of performing compensation transformation on said duplicate digitized signal comprises compensating a single parameter.

148. A method of flow cytometry as described in claim 147, wherein said step of compensating a single parameter comprises compensating a serial digitized analog signal.

149. A method of flow cytometry as described in claim 148, wherein said step of compensating said serial analog signal comprises minimizing variations selected from the group consisting of phase, or shape.

150. A method of flow cytometry as described in claim 146, wherein said step of performing compensation transformation on said signal comprises compensating at least two different parameters.

151. A method of flow cytometry as described in claim 150, wherein said step of compensating at least two different parameters comprises minimizing characteristics shared by said at least two different parameters.

152. A method of flow cytometry as described in claim 151, wherein said step of minimizing characteristics shared by said at least two different parameters comprises reducing spectrum overlap.

153. A flow cytometer, comprising:

a. a fluid stream;

b. an occurrence incident to said fluid stream;

c. a sensor responsive to said occurrence incident to said fluid stream;

d. at least one signal generator coupled to said sensor;

e. signal data generated by said at least one signal generator;

f. a memory element to store said signal data;

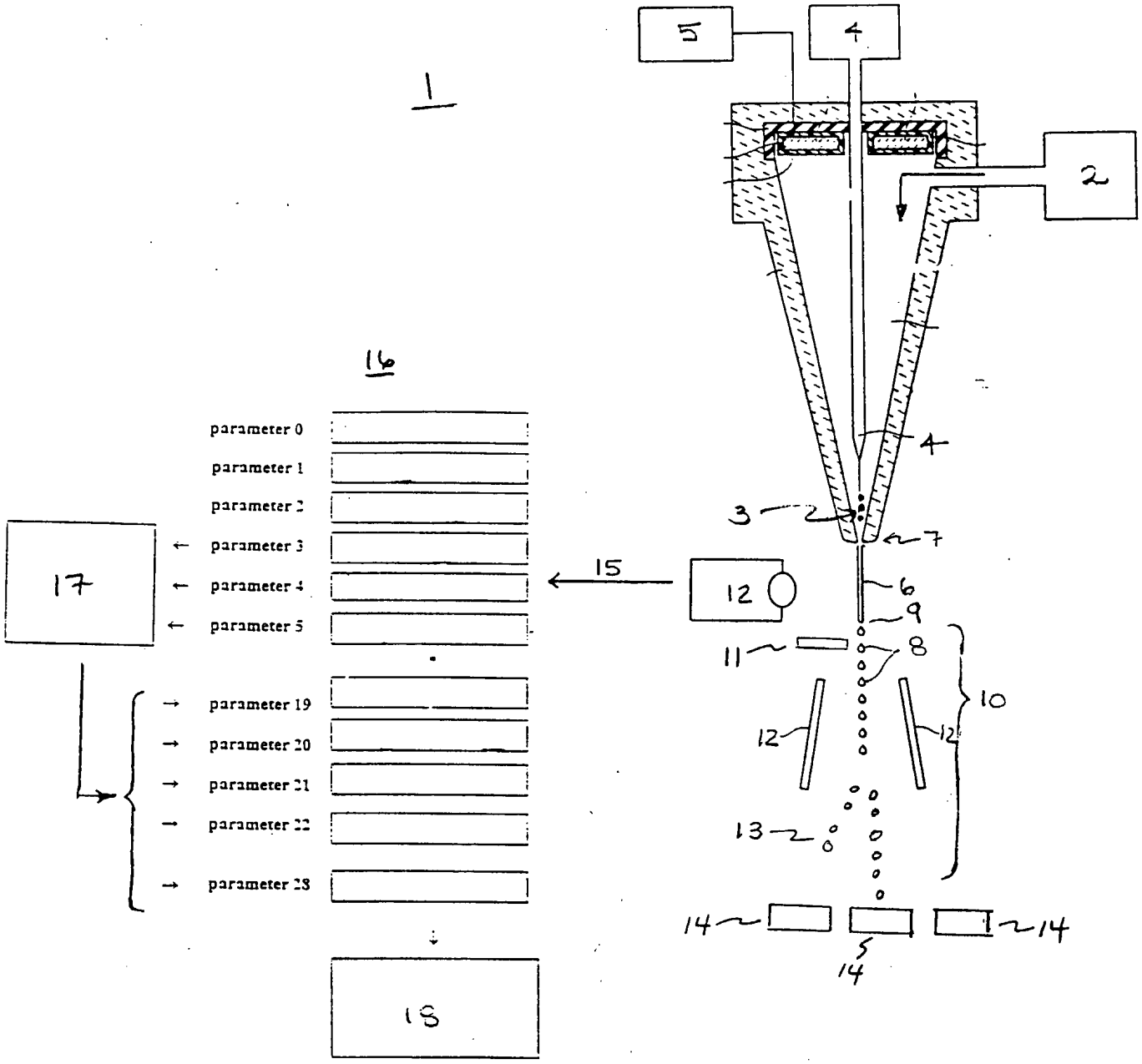
g. a stored signal data retreival element;

h. a stored signal duplicator; and

i. at least one signal processor responsive to a duplicated signal.

154. Methods substantially as described hereinbefore and with reference to any of the accompanying examples.

155. Apparatuses substantially as described hereinbefore and with reference to any of the accompanying examples.


1
5
4
2
16
parameter 0
parameter 1
parameter 2
parameter 3
parameter 4
parameter 5
parameter 19
parameter 20
parameter 21
parameter 22
parameter 28
17
15
12
18
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8
10
13
14

FIG 1

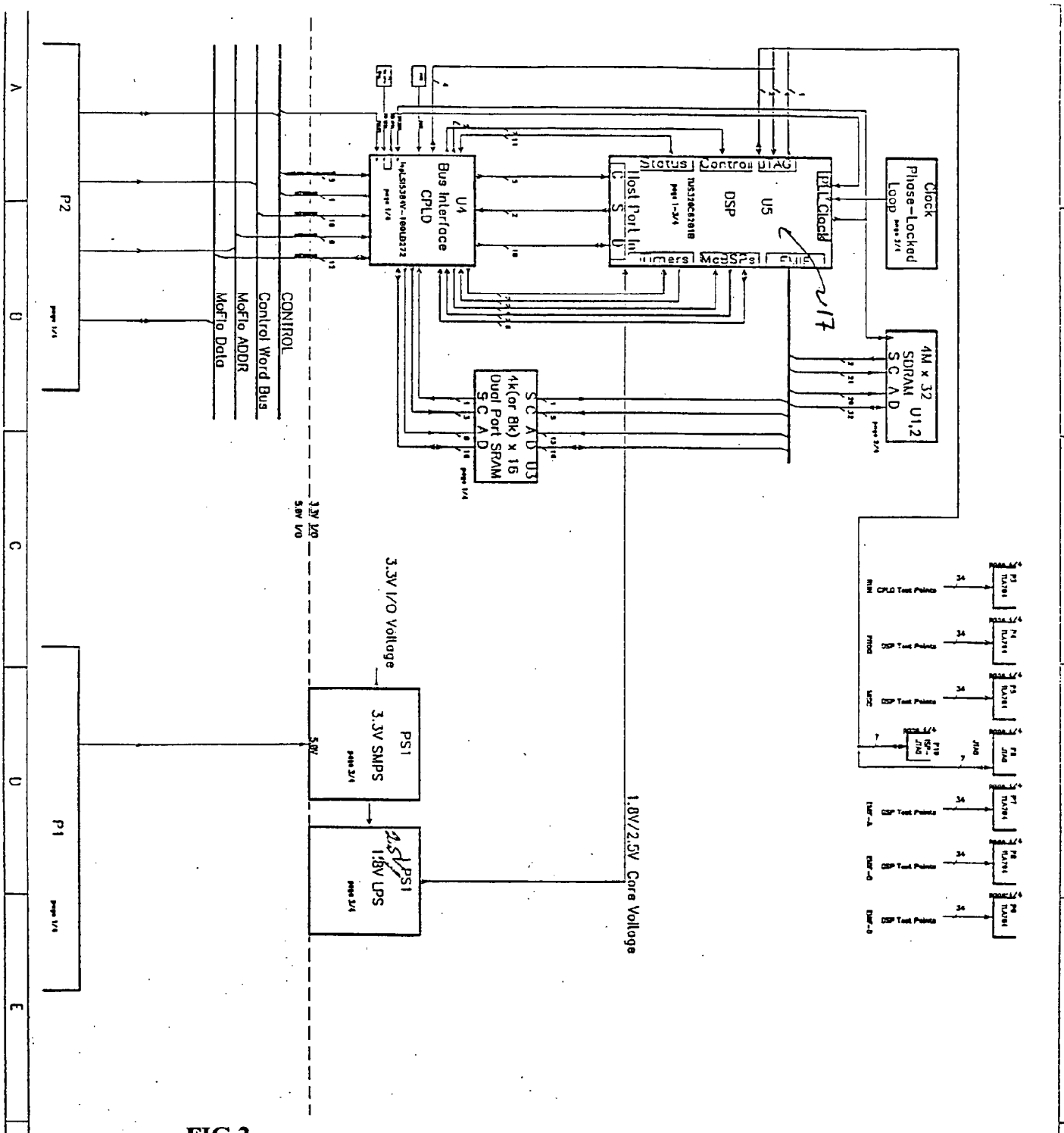
Clock
Phase-Locked
Loop
4M x 32
SDRAM U12
S C A D
U4
Bus Interface
CPLD
DSP
U5
Status
Control
JTAG
Host Port Int
Timers
McBSPs
EMIF
PLL Clock
S C A D U3
4k(or 8k) x 16
Dual Port SRAM
S C A D
P2
P1
CONTROL
Control Word Bus
MoFlo ADDR
MoFlo Data
3.3V I/O Voltage
PS1
3.3V SMPS
PS1
1.8V LPS
1.8V/2.5V Core Voltage

FIG 2

ATTACHMENT A

```
;*****************************************************************************
;* FUNCTION NAME: _cpm_run                                                   *
;*                                                                           *
;*   Regs Modified     : A0,A1,A2,A3,A4,A5,A6,A7,A8,A9,A10,A11,A12,A13,B0,B1, *
;*                       B2,B3,B4,B5,B6,B7,B8,B9,B10,B11,B12,B13,DP          *
;*   Regs Used         : A0,A1,A2,A3,A4,A5,A6,A7,A8,A9,A10,A11,A12,A13,B0,B1, *
;*                       B2,B3,B4,B5,B6,B7,B8,B9,B10,B11,B12,B13,DP          *
;*****************************************************************************
_cpm_run:
;** ---------------------------------------------------------------------*
;
; _cpm_run  .proc P0c, P1c, P2c, P3c, P4c, P5c, P6c, P7c
;           .reg  ctable0, ctable1, map, tmp, counter, idMask
          MVK     .S1     _srcAdr,A12  ; |62|

          MVK     .S1     0xfff,A13    ; |62|
||        MVK     .S2     _ctable,B3   ; |62|  Address pointer to coefficient table

          MVK     .S2     0x8,B2       ; |62|  Prime the counter
||        MVKH    .S1     _srcAdr,A12  ; |62|

          LDW     .D1T1   *A12,A12     ; |62|
||        MVKH    .S2     _ctable,B3   ; |62|
||        ZERO    .L1     A10          ; |62|   P6
||        MVK     .S1     _exp_map,A3  ; |62|  Map to map Pn -> exp(Pn/A)

          ADDK    .S2     0x4,B3       ; |62|
||        MV      .L1X    B3,A2        ; |62|
||        MVKH    .S1     0x0,A13      ; |62|
||        ZERO    .L2     B10          ; |62|   P7
||        ZERO    .D2     B8           ; |62|   P5
||        ZERO    .D1     A6           ; |62|   P2

          MVC     .S2     CSR,B12      ; |62|
||        MVKH    .S1     _exp_map,A3  ; |62|
||        ZERO    .L2     B6           ; |62|   P3
||        ZERO    .D2     B4           ; |62|   P1 set to zero
||        ZERO    .L1     A4           ; |62|   P0 Running sum of computed parameters
||        ZERO    .D1     A8           ; |62|   P4

          AND     .L2     -2,B12,B5    ; |62|

          MVC     .S2     B5,CSR       ; |62|
||        SUB     .L2     B2,4,B2      ; |62|

;** ---------------------------------------------------------------------*
L10:       ; PIPED LOOP PROLOG
; cpm_loop  .trip 8
          LDW     .D1T1   *A12++,A7    ; |62|  Load MOFLO parameter Pn
          NOP             4
          LDW     .D1T1   *A2++(16),A0 ; |62|  Get columnwise compensation coefficients

          AND     .S1     A13,A7,A7    ; |62|
||        LDW     .D1T1   *A12++,A7    ; a|62|  Load MOFLO parameter Pn

          LDW     .D2T2   *B3++(16),B0 ; |62|  Coefficients are loaded low/high low high etc.
||        LDW     .D1T1   *+A3[A7],A5  ; |62|  MOFLO Pn -> exp(Pn/A)

          LDW     .D1T1   *-A2(8),A1   ; |62|
||        LDW     .D2T2   *-B3(8),B1   ; |62|

          NOP             2
          LDW     .D1T1   *A2++(16),A0 ; a|62|  Get columnwise compensation coefficients

          MPYHSLU .M2X    B0,A5,B7     ; |62|
||        MPYHSLU .M1     A0,A5,A7     ; |62|
||        AND     .S1     A13,A7,A7    ; a|62|
||        LDW     .D1T1   *A12++,A7    ; aa|62|  Load MOFLO parameter Pn

;** ---------------------------------------------------------------------*
cpm_loop:        ; PIPED LOOP KERNEL

   [ B2]  B       .S2     cpm_loop     ;  |62|
||        MPYSU   .M2X    B1,A5,B9     ; |62|
||        MPYHSLU .M1     A1,A5,A11    ; |62|
||        LDW     .D2T2   *B3++(16),B0 ; a|62|  Coefficients are loaded low/high low high etc.
||        LDW     .D1T1   *+A3[A7],A5  ; a|62|  MOFLO Pn -> exp(Pn/A)
```

```
          SUB     .L2     B6,B7,B6     ; |62|
||        MPYHSLU .M2X    B1,A5,B11    ; |62|
||        MPYSU   .M1     A1,A5,A9     ; |62|
||        LDW     .D1T1   *-A2(8),A1   ; a|62|
||        LDW     .D2T2   *-B3(8),B1   ; a|62|

          MPYSU   .M1     A0,A5,A5     ; |62|  Evaluate -log(1 - Cn1)*exp(Pn/A)
||        SUB     .L1X    A10,B9,A10   ; |62|
||        MV      .L2X    A5,B5        ; |62|

          SUB     .S2     B10,B11,B10  ; |62|
||        MV      .L2X    A7,B5        ; |62|
||        MPYSU   .M2     B0,B5,B5     ; |62|
||        SUB     .L1     A8,A9,A8     ; |62|

          SUB     .L1     A4,A5,A4     ; |62|  Running sum P0c -= log(1 - Cn1)*exp(Pn/A)
||        SUB     .L2X    B8,A11,B8    ; |62|
||        SUB     .S2     B4,B5,B4     ; |62|
||        LDW     .D1T1   *A2++(16),A0 ; aa|62|  Get columnwise compensation coefficients

          SUB     .L1X    A6,B5,A6     ; |62|
|| [ B2]  SUB     .L2     B2,0x1,B2    ; a|62|
||        MPYHSLU .M2X    B0,A5,B7     ; a|62|
||        MPYHSLU .M1     A0,A5,A7     ; a|62|
||        AND     .S1     A13,A7,A7    ; aa|62|
||        LDW     .D1T1   *A12++,A7    ; aaa|62|  Load MOFLO parameter Pn

;** ---------------------------------------------------------------------------*
L12:      ; PIPED LOOP EPILOG

          MPYSU   .M2X    B1,A5,B9     ; a|62|
||        MPYHSLU .M1     A1,A5,A11    ; a|62|
||        LDW     .D2T2   *B3++(16),B0 ; aa|62|  Coefficients are loaded low/high low high etc.
||        LDW     .D1T1   *+A3[A7],A5  ; aa|62|  MOFLO Pn -> exp(Pn/A)

          SUB     .L2     B6,B7,B6     ; a|62|
||        MPYHSLU .M2X    B1,A5,B11    ; a|62|
||        MPYSU   .M1     A1,A5,A9     ; a|62|
||        LDW     .D1T1   *-A2(8),A1   ; aa|62|
||        LDW     .D2T2   *-B3(8),B1   ; aa|62|

          MPYSU   .M1     A0,A5,A5     ; a|62|  Evaluate -log(1 - Cn1)*exp(Pn/A)
||        SUB     .L1X    A10,B9,A10   ; a|62|
||        MV      .L2X    A5,B5        ; a|62|

          SUB     .S2     B10,B11,B10  ; a|62|
||        MV      .L2X    A7,B5        ; a|62|
||        MPYSU   .M2     B0,B5,B5     ; a|62|
||        SUB     .L1     A8,A9,A8     ; a|62|

          SUB     .L1     A4,A5,A4     ; a|62|  Running sum P0c -= log(1 - Cn1)*exp(Pn/A)
||        SUB     .L2X    B8,A11,B8    ; a|62|
||        SUB     .S2     B4,B5,B4     ; a|62|
||        LDW     .D1T1   *A2++(16),A0 ; aaa|62|  Get columnwise compensation coefficients

          SUB     .L1X    A6,B5,A6     ; a|62|
||        MPYHSLU .M2X    B0,A5,B7     ; aa|62|
||        MPYHSLU .M1     A0,A5,A7     ; aa|62|
||        AND     .S1     A13,A7,A7    ; aaa|62|

          MPYSU   .M2X    B1,A5,B9     ; aa|62|
||        MPYHSLU .M1     A1,A5,A11    ; aa|62|
||        LDW     .D2T2   *B3++(16),B0 ; aaa|62|  Coefficients are loaded low/high low high etc.
||        LDW     .D1T1   *+A3[A7],A5  ; aaa|62|  MOFLO Pn -> exp(Pn/A)

          SUB     .L2     B6,B7,B6     ; aa|62|
||        MPYHSLU .M2X    B1,A5,B11    ; aa|62|
||        MPYSU   .M1     A1,A5,A9     ; aa|62|
||        LDW     .D1T1   *-A2(8),A1   ; aaa|62|
||        LDW     .D2T2   *-B3(8),B1   ; aaa|62|
```

```
           MPYSU   .M1     A0,A5,A5     ; aa|62|  Evaluate -log(1 - Cn1)*exp(Pn/A)
||         SUB     .L1X    A10,B9,A10   ; aa|62|
||         MV      .L2X    A5,B5        ; aa|62|

           SUB     .S2     B10,B11,B10  ; aa|62|
||         MV      .L2X    A7,B5        ; aa|62|
||         MPYSU   .M2     B0,B5,B5     ; aa|62|
||         SUB     .L1     A8,A9,A8     ; aa|62|

           SUB     .L1     A4,A5,A4     ; aa|62|  Running sum POc -= log(1 - Cn1)*exp(Pn/A)
||         SUB     .L2X    B8,A11,B8    ; aa|62|
||         SUB     .S2     B4,B5,B4     ; aa|62|

           SUB     .L1X    A6,B5,A6     ; aa|62|
||         MPYHSLU .M2X    B0,A5,B7     ; aaa|62|
||         MPYHSLU .M1     A0,A5,A7     ; aaa|62|

           MPYSU   .M2X    B1,A5,B9     ; aaa|62|
||         MPYHSLU .M1     A1,A5,A11    ; aaa|62|

           SUB     .L2     B6,B7,B6     ; aaa|62|
||         MPYHSLU .M2X    B1,A5,B11    ; aaa|62|
||         MPYSU   .M1     A1,A5,A9     ; aaa|62|

           MPYSU   .M1     A0,A5,A5     ; aaa|62|  Evaluate -log(1 - Cn1)*exp(Pn/A)
||         SUB     .L1X    A10,B9,A10   ; aaa|62|
||         MV      .L2X    A5,B5        ; aaa|62|

           SUB     .S2     B10,B11,B10  ; aaa|62|
||         MV      .L2X    A7,B5        ; aaa|62|
||         MPYSU   .M2     B0,B5,B5     ; aaa|62|
||         SUB     .L1     A8,A9,A8     ; aaa|62|

           SUB     .L1     A4,A5,A4     ; aaa|62|  Running sum POc -= log(1 - Cn1)*exp(Pn/A)
||         SUB     .L2X    B8,A11,B8    ; aaa|62|
||         SUB     .S2     B4,B5,B4     ; aaa|62|

           SUB     .L1X    A6,B5,A6     ; aaa|62|
;** --------------------------------------------------------------------------*

           LDW     .D1T1   *--A12(8),A11 ; |62|  Load all MOFLO uncompensated parameters
||         MV      .L2X    A12,B12      ; |62|
||         MVC     .S2     B12,CSR      ; |62|

           LDW     .D1T1   *--A12(8),A9 ; |62|
||         ADDK    .S2     0x4,B12      ; |62|

           LDW     .D1T1   *--A12(8),A7 ; |62|
||         LDW     .D2T2   *--B12(8),B11 ; |62|

           LDW     .D1T1   *--A12(8),A5 ; |62|
||         LDW     .D2T2   *--B12(8),DP ; |62|

           LDW     .D2T2   *--B12(8),B7 ; |62|
           LDW     .D2T2   *--B12(8),B5 ; |62|
           MVK     .S1     _inv_exp,A2  ; |62|  Load inverse map
           MVKH    .S1     _inv_exp,A2  ; |62|
           AND     .L1     A13,A5,A5    ; |62|

           LDW     .D1T1   *+A2[A5],A0  ; |62|  Map to exp(-Pn/A)
||         MV      .L2X    A2,B2        ; |62|

           AND     .L2X    A13,B5,B5    ; |62|
           LDW     .D2T2   *+B2[B5],B0  ; |62|

           NORM    .L1     A4,A12       ; |62|  Pnc is normalized
||         AND     .S1     A13,A7,A7    ; |62|

           LDW     .D1T1   *+A2[A7],A1  ; |62|
||         SHL     .S1     A4,A12,A4    ; |62|  Bit shift is saved
```

```
           MPYH    .M1     A4,A0,A4     ; |62|  Pnc x exp(-Pn/A)
||         NORM    .L2     B4,B12       ; |62|
||         AND     .S2X    A13,B7,B7    ; |62|

           ADD     .L1     A0,A12,A12   ; |62|  Add to exponent part of exp(-Pn/A)
||         SHL     .S2     B4,B12,B4    ; |62|
||         LDW     .D2T2   *+B2[B7],B1  ; |62|
||         AND     .S1     A13,A9,A9    ; |62|

           MPYH    .M2     B4,B0,B4     ; |62|
||         NORM    .L1     A6,A12       ; |62|
||         SHR     .S1     A4,A12,A4    ; |62|  Shift the result

           SHL     .S1     A6,A12,A6    ; |62|
||         ADD     .L2     B0,B12,B12   ; |62|
||         LDW     .D1T1   *+A2[A9],A3  ; |62|
||         AND     .S2X    A13,DP,DP    ; |62|

           MPYH    .M1     A6,A1,A6     ; |62|
||         NORM    .L2     B6,B12       ; |62|
||         SHR     .S2     B4,B12,B4    ; |62|

           ADD     .L1     A1,A12,A12   ; |62|
||         SHL     .S2     B6,B12,B6    ; |62|
||         LDW     .D2T2   *+B2[DP],B3  ; |62|

           MPYH    .M2     B6,B1,B6     ; |62|
||         NORM    .L1     A8,A12       ; |62|
||         SHR     .S1     A6,A12,A6    ; |62|
||         AND     .L2X    A13,B11,B11  ; |62|

           SHL     .S1     A8,A12,A8    ; |62|
||         ADD     .L2     B1,B12,B12   ; |62|
||         ZERO    .S2     B2           ; |62|
||         LDW     .D2T2   *+B2[B11],B13 ; |62|
||         AND     .L1     A13,A11,A11  ; |62|

           MVK     .S1     0xfff,A3     ; |62|
||         ADD     .L1     A3,A12,A12   ; |62|
||         MPYH    .M1     A8,A3,A8     ; |62|
||         NORM    .L2     B8,B12       ; |62|
||         SHR     .S2     B6,B12,B6    ; |62|
||         ADD     .D2     B5,B4,B4     ; |62|
||         LDW     .D1T1   *+A2[A11],A13 ; |62|

           MVKH    .S1     0xfff,A3     ; |62|
||         SHL     .S2     B8,B12,B8    ; |62|
||         CMPLT   .L2     B4,B2,B1     ; |62|
||         ADD     .L1     A5,A4,A4     ; |62|  Add Pn
||         ZERO    .D1     A2           ; |62|

           MV      .L2X    A3,B3        ; |62|
||         ADD     .S2     B3,B12,B12   ; |62|
|| [ B1]   ZERO    .D2     B4           ; |62|
||         MPYH    .M2     B8,B3,B8     ; |62|
||         CMPLT   .L1     A4,A2,A1     ; |62|  Saturate values negative to zero

           NORM    .L1     A10,A12      ; |62|
||         CMPGT   .L2X    B4,A3,B1     ; |62|
||         SHR     .S1     A8,A12,A8    ; |62|
|| [ A1]   ZERO    .D1     A4           ; |62|
||         ADD     .S2     B7,B6,B6     ; |62|

           CMPLT   .L2     B6,B2,B1     ; |62|
||         CMPGT   .L1     A4,A3,A1     ; |62|
|| [ B1]   MV      .S2     B3,B4        ; |62|
||         ADD     .S1     A7,A6,A6     ; |62|

           CMPLT   .L1     A6,A2,A1     ; |62|
|| [ A1]   MV      .D1     A3,A4        ; |62|
```

```
||          NORM    .L2     B10,B12         ; |62|
||          SHR     .S2     B8,B12,B8       ; |62|
||          MVK     .S1     _output_data,A5 ; |62|

   [ A1]    ZERO    .L1     A6              ; |62|
||          SHL     .S2     B10,B12,B10     ; |62|
|| [ B1]    ZERO    .L2     B6              ; |62|
||          SHL     .S1     A10,A12,A10     ; |62|

||          MPYH    .M2     B10,B13,B10     ; |62|
||          CMPGT   .L1     A6,A3,A1        ; |62|
||          MPYH    .M1     A10,A13,A10     ; |62|
||          ADD     .S2     DP,B8,B8        ; |62|
||          CMPGT   .L2     B6,B3,B1        ; |62|
||          ADD     .D1     A9,A8,A8        ; |62|
||          MVKH    .S1     _output_data,A5 ; |62|

||          CMPLT   .L2     B8,B2,B1        ; |62|
|| [ B1]    MV      .S2     B3,B6           ; |62|
||          ADD     .D2     B13,B12,B12     ; |62|
||          CMPLT   .L1     A8,A2,A1        ; |62|
||          ADD     .S1     A13,A12,A12     ; |62|
|| [ A1]    MV      .D1     A3,A6           ; |62|

   [ B1]    ZERO    .L2     B8              ; |62|
||          SHR     .S1     A10,A12,A10     ; |62|
||          SHR     .S2     B10,B12,B10     ; |62|
|| [ A1]    ZERO    .L1     A8              ; |62|

||          ADD     .S1     A11,A10,A10     ; |62|
||          CMPGT   .L2     B8,B3,B1        ; |62|
||          CMPGT   .L1     A8,A3,A1        ; |62|
||          ADD     .D2     B11,B10,B10     ; |62|
||          MV      .S2X    A5,B5           ; |62|

||          CMPLT   .L1     A10,A2,A1       ; |62|
||          CMPLT   .L2     B10,B2,B1       ; |62|
|| [ B1]    MV      .D2     B3,B8           ; |62|
|| [ A1]    MV      .S1     A3,A8           ; |62|
||          ADDK    .S2     0x4,B5          ; |62|

   [ A1]    ZERO    .L1     A10             ; |62|
||          STW     .D1T1   A4,*A5++(8)     ; |62|
||          STW     .D2T2   B4,*B5++(8)     ; |62|
|| [ B1]    ZERO    .L2     B10             ; |62|

||          CMPGT   .L1     A10,A3,A1       ; |62|
||          CMPGT   .L2     B10,B3,B1       ; |62|
||          STW     .D2T2   B6,*B5++(8)     ; |62|
||          STW     .D1T1   A6,*A5++(8)     ; |62|

   [ B1]    MV      .L2     B3,B10          ; |62|
|| [ A1]    MV      .L1     A3,A10          ; |62|
||          STW     .D1T1   A8,*A5++(8)     ; |62|
||          STW     .D2T2   B8,*B5++(8)     ; |62|

||          STW     .D2T2   B10,*B5++(8)    ; |62|
||          STW     .D1T1   A10,*A5++(8)    ; |62|

;** --------------------------------------------------------------------------*
cpm_end:

; cpm_end   .endproc P0c, P1c, P2c, P3c, P4c, P5c, P6c, P7c
```

In the foregoing, the parallel bars are operations performed in one clock. The total number of clocks, ignoring the stack saving before and after which are imbedded to test the code from a C routine counts to 106 clocks or 540 ns. This gives the total MoFlo® data words as 540/150 (150 is the time between MOFLO data words) = 3.5 MOFLO data words.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/41372

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :B07C 5/02
US CL :702/45, 21, 29; 209/3.1, 564
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

USPTO APS EAST
search terms: cytometry, particles, fluid, flow, transformation, compensation, processor, microprocessor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,367,474 A (AUER et al) 22 November 1994 (22.11.1994), Abstract; figs. 2-4; col. 5, lines 31-43; and col. 19, lines 25-49.	45-47, 61, 78-79, 91-93, 100-102, 126
X	US 4,987,539 A (MOORE et al) 22 January 1991 (22.01.1991), Abstract; figs. 4, 7A, 7B; col. 2, lines 13-15, 31; col. 4, lines 33-38; col. 15, lines 60-68; and col. 16, lines 1-12.	78-84, 99-102, 126
X	US 5,199,576 A (CORIO et al) 06 April 1993, (06.04.1993) Abstract; figs. 2, 3A, 3D, 4, 5; and col. 16, lines 23-67.	78, 79
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A		1-44, 48-60, 62-77, 85-90, 94-98, 103-125, 127-153

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 20 FEBRUARY 2001	Date of mailing of the international search report 07 MAR 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer HAL D. WACHSMAN Telephone No. (703) 305-9788

Form PCT/ISA/210 (second sheet) (July 1998)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/41372

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 154, 155
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Please See Extra Sheet.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/41372

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

702/45, 21, 29, 22, 23, 25-28, 30-32, 49, 50, 55, 57, 85, 100, 104, 127, 128, 179, 183, 189, 198, FOR 103, FOR 104, FOR 115-FOR 119, FOR 121, FOR 127, FOR 128, FOR 134, FOR 139-FOR 141, FOR 156-FOR 163, FOR 170, FOR 171; 209/3.1, 564, 3, 576, 579, 906; 700/266, 271, 273, 281, 282, 285; 377/21; 436/50, 17, 10; 356/39; 422/50, 62, 67, 68.1, 73; 73/863.21, 863.22, 865.5, 861.39, 861.41, 861.02, 861.03, 861.04

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE
2. Where no meaningful search could be carried out, specifically:

Claim 154 refers to methods substantially as described hereinbefore and with reference to any of the accompanying examples. Thus it cannot be ascertained exactly which method is being referred to and the claim does not point out the exact example being referred to and does not incorporate such an example in the claim. These same type of problems also occur in claim 155 which in similiar fashion refers to apparatuses substantially described hereinbefore.